Characterizing Emotion Regulation Deficits in Psychiatric Disorders using Psychophysiological, Neurobiological, and Genetic Methods

---

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde
(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

Michael M. Havranek

von

Luzern LU

Promotionskomitee

Prof. Dr. Klaas Prüssmann (Vorsitz)

Prof. Dr. med. Erich Seifritz (Leitung der Dissertation)

Zürich, 2016

Contents

Summary

Emotion regulation includes all processes by which an individual influences which emotions are experienced, how and when they are experienced, and how they are expressed. These processes are often disturbed in psychiatric patients. According to the diathesis-stress model, psychiatric disorders develop if environmental factors (such as stressors) exceed a coping threshold defined by an individual's psychological, neurobiological, and genetic predispositions (coined as individual vulnerability factors). In this PhD project, we investigated whether emotion regulation deficits as well develop as a result of an interaction between environmental and individual factors. For that, we designed three experiments during which participants were directed to manage their emotions while we systematically varied environmental factors and identified critical individual predispositions of the participants. In study A, we confronted healthy participants with uncontrollable and unpredictable stress and assessed how participants' subclinical symptoms of anxiety and depression moderated their psychophysiological anxiety response. In study B, we administered gamma-hydroxybutyrate (GHB) to a sample of healthy participants and assessed how the drug influenced their sexual arousal and which neural underpinnings mediated this effect. In study C, we investigated how chronic cocaine use and three α_{2A}-adrenergic receptor (*ADRA2A*) polymorphisms mediated deficits in delaying gratification (coined as delay discounting). In study A, we found that the uncontrollability and unpredictability of stressors determined participants' anxiety response in interaction with their subclinical symptoms of anxiety and depression. In study B, we revealed that GHB lowered the threshold for sexual arousal by activating a sex-cue specific neuronal network. In study C, we showed that cocaine consumption determined deficits in delay discounting in interaction with three polymorphisms in the *ADRA2A* gene. In conclusion, the three studies of this PhD project provided evidence that specific emotion regulation deficits develop from an interaction of specific environmental and individual factors consistent with the diathesis-stress model. As a consequence, we propose that the concept of emotion regulation should be broadened for widespread application in psychopathological research.

Summary in German

Emotionsregulation beinhaltet alle Prozesse, mit welchen eine Person beeinflusst, welche Gefühle erlebt werden, wie und wann sie erlebt werden und wie sie ausgedrückt werden. Diese Prozesse sind oft gestört in psychiatrischen Patienten. Gemäss dem Diathesis-Stress Modell entstehen psychiatrische Störungen, wenn Umwelteinflüsse (wie z.B. Stress) die durch individuelle psychologische, neurobiologische und genetische Veranlagungen (bezeichnet als individuelle Faktoren) bestimmten Bewältigungsstrategien einer Person übersteigen. In diesem PhD Projekt untersuchten wir, ob auch Emotionsregulationsstörungen als Resultat einer Interaktion von Umweltfaktoren und individuellen Faktoren entstehen. Dazu entwickelten wir drei Experimente, bei welchen Versuchspersonen ihre Gefühle regulieren mussten während wir systematisch die Umweltfaktoren variierten und entscheidende individuelle Faktoren identifizierten. In Studie A konfrontierten wir gesunde Versuchspersonen mit unkontrollierbarem und unvorhersehbarem Stress und beurteilten, wie ihre subklinischen Angst- und Depressions-Symptome die Angstreaktion beeinflussten. In Studie B verabreichten wir gesunden Probanden Gammahydroxybutyrat (GHB) und beurteilten, wie das Medikament ihre sexuelle Erregung beeinflusste und welche neuronalen Netzwerke diesen Effekt vermittelten. In Studie C untersuchten wir, wie chronischer Kokainkonsum und drei α_{2A}-Adrenorezeptor-Polymorphismen die Fähigkeit Belohnung aufzuschieben beeinträchtigen. In Studie A fanden wir, dass die Unkontrollierbarkeit und Unvorhersehbarkeit der Stressoren die Angstreaktion der Versuchspersonen in Interaktion mit ihren subklinischen Angst- und Depressions-Symptomen bestimmten. In Studie B zeigten wir auf, dass GHB den Schwellenwert der sexuellen Erregung senkte, indem es ein Sex-spezifisches neuronales Netzwerk aktivierte. In Studie C demonstrierten wir, dass chronischer Kokainkonsum in Interaktion mit den drei α_{2A}-Adrenorezeptor-Polymorphismen zu Störungen des Belohnungsaufschubes führte. Die drei Studien dieses PhD Projekts lieferten Nachweis dafür, dass spezifische Emotionsregulationsstörungen gemäss dem Diathesis-Stress Modell als Resultat einer Interaktion von spezifischen Umweltfaktoren und individuellen Faktoren entstehen. Folglich schlagen wir vor, dass das Konstrukt der Emotionsregulation erweitert werden sollte, um es zur breitgefächerten Anwendung in der psychopathologischen Forschung einzusetzen.

1 Introduction

This chapter will define fundamental terms, introduce the topic, and deduce the hypothesis and methods used in this PhD project.

1.1 Emotion regulation deficits in psychiatric patients

Emotions are defined as coordinated sets of responses that occur when an individual evaluates a situation as offering opportunities or challenges (Lazarus, 1993). These response sets involve cognitive, behavioral, physiological, and neural mechanisms, and aim to orchestrate the best possible response to the specific event (Lang, 1995). The subjective experience of an emotion is called *feeling*. In contrast to emotions and feelings, *moods* refer to longer-lasting sensations. Finally, *affect* is an encompassing term which comprises emotions, feelings, and moods. However, in everyday language, the terms emotion, feeling, and mood are used interchangeably (Gross, 2014).

Emotion regulation is defined as including all processes by which individuals influence which emotions they experience, when they experience them, and how they experience and express them. These mechanisms may either be conscious and controlled or unconscious and automatic (Thompson, 1994). Most often, individuals attempt to decrease negative emotions such as anger, sadness, or anxiety (Gross et al., 2006) and increase positive emotions such as love, interest, or joy (Quoidbach et al., 2010). However, emotion regulation involves all changes in the latency, rise time, magnitude, duration, and offset of emotions, as well as changes in how emotional responses are interrelated (for example when feelings occur without the accompanying behaviors) (Gross, 1998).

Although the concept of emotion regulation has gained great scientific interest throughout the last two decades, most studies focused almost exclusively on investigating a specific subset of emotion regulation strategies called reappraisal and suppression. *Reappraisal* is a cognitive strategy in which an individual reinterprets a situation in a way that alters the emotional response, while *suppression* is an emotional strategy in which an individual decreases emotional behavior when emotionally aroused (Webb et al., 2012). There are a multitude of publications available on reappraisal and suppression, including studies identifying individual differences in their use and effect, neuroscientific studies pointing to different neural underpinnings involved, and even a few genetic studies hinting at different molecular mediators (Gross & Thompson, 2007).

For instance, it was reported that reappraisal leads to decreased levels of negative emotions, slightly decreased psychophysiological arousal via the sympathetic nervous system, and decreased activation in the amygdala and the ventral striatum. Suppression, on the other hand, leads to decreased positive (instead of negative) emotions, increased responses of the sympathetic nervous system, and greater activation in the amygdala (Gross & Thompson, 2007). A few genetic studies found evidence that the polymorphism in the serotonin-transporter gene promoter region (*5-HTTLPR*) may be involved in mediating reappraisal (Miu et al., 2013), while a polymorphism in the oxytocin receptor (*OXTR*) gene (rs53576) seems to be involved in mediating suppression (Kim et al., 2011). However, due to the strict focus of researchers on the strategies of reappraisal and suppression, many other aspects of the broad topic of emotion regulation have been neglected so far (Berking & Wupperman, 2012).

An important topic for this PhD project is the development of emotion regulation deficits in psychiatric disorders. Interestingly, there is a significant amount of evidence showing an association between emotion regulation deficits (i.e., the inability to effectively regulate emotions) and mental illness across most psychiatric disorders (American Psychiatric Association, 1994). Among the psychiatric disorders, the impact of emotion regulation deficits appears most significantly in anxiety disorders, depression, and substance disorders.

In the case of anxiety disorders, studies in non-clinical samples showed that emotion regulation skills (Wirtz et al., 2014), emotion regulation deficits (Berking et al., 2008), and beliefs regarding successful future emotion regulation (Kassel et al., 2007) predicted subsequent anxiety symptom severity in periods ranging from two weeks to five years. Several studies in clinical samples further showed that compared to healthy controls, patients suffering from generalized anxiety disorder, panic disorders, social anxiety disorders, and post-traumatic stress disorders display poorer understanding of emotions, greater negative reactions to emotions, and less successful management of emotions by using avoidance strategies rather than emotion regulation to cope with subjectively difficult situations (Davey et al., 1995, Kraaij et al., 2003, McLaughlin et al., 2007, Naragon-Gainey, 2010, Tull & Roemer, 2007, Weiss et al., 2012).

Patients suffering from depression also exhibit problems identifying their emotions, accepting negative emotions, and successfully managing their emotions (Campbell-Sills et al., 2014, Ehring et al., 2008, Rude & McCarthy, 2003). In addition, depressed individuals have been shown to use unsuccessful attempts to manage negative emotions, such as ruminating, catastrophizing, or suppressing (Aldao et al., 2010, Berking & Wupperman, 2012). Longitudinal studies in clinical samples found that patients' use of maladaptive regulation strategies predicted depressive symptoms, while patients' beliefs in their ability to efficiently

manage their emotions predicted subsequent reductions in depressive symptoms (Aldao & Nolen-Hoeksema, 2012, Kassel et al., 2007).

In substance disorders, the use of alcohol and drugs is in itself a maladaptive attempt to regulate negative emotions (Baker et al., 2004). Because of this, the ability to effectively regulate one's emotions is necessary for sobriety, and patients suffering from substance disorders often report emotion regulation deficits compared to healthy controls (Berking et al., 2011). Consistently, longitudinal studies showed that negative emotions predicted future levels of alcohol consumption while deficits in emotion regulation predicted relapse during and after treatment for alcohol dependence (Berking et al., 2011, Gamble et al., 2010).

1.2 Objective, aim and hypothesis of this PhD project

The objective of this PhD project was to characterize emotion regulation deficits in several psychiatric disorders using psychophysiological, neurobiological, and genetic research methods. However, to deduce the specific aim and hypothesis of this project, we must first state its two fundamental assumptions:

1. Most studies on emotion regulation have primarily focused on the strategies of reappraisal and suppression, yet have neglected other important topics of emotion regulation. Due to this, a basic understanding of the causes and underlying factors of emotion regulation deficits in psychiatric disorders is still absent (Berking & Wupperman, 2012). In our opinion, the concept of emotion regulation should be used in a broader sense, because it overlaps with other models describing control over emotions that are important in psychopathology. In accordance with our opinion, recent efforts have been made to integrate emotion regulation and emotional intelligence (a concept very similar to emotion regulation, but primarily focusing on individual differences) (Peña-Sarrionandia et al., 2015). We believe, however, that this is just the tip of the iceberg and that emotion regulation should be used as an encompassing term including several constructs that have previously been studied separately.

2. According to the popular diathesis-stress model, psychopathologies or more general behaviors of any kind develop as a consequence of an interaction between environmental factors (e.g., stressors) and individual psychological, neurobiological, and genetic predispositions (from now on referred to as individual factors)(Ingram & Luxton, 2005). This model states that psychiatric disorders develop if environmental factors exceed a coping threshold defined by an individual's

predispositions (Lazarus, 1993). Although the diathesis-stress model has been used to explain the formation of diverse psychiatric conditions, it has not been used to explain the development of emotion regulation deficits which may underlie these conditions. Thus, to the best of our knowledge, the environmental and individual factors facilitating the formation of emotion regulation deficits in psychopathological disorders remain unclear to this date (Berking & Wupperman, 2012).

Consistently, the aim of this PhD project was to use different etiological models for specific psychiatric disorders, such as the learned helplessness model for depression or the proposed delay discounting endophenotype for cocaine addiction (both models are explained below), as specific examples of emotion regulation deficits to make inferences about the underlying causes of emotion regulation deficits in psychiatric patients in general.

Thus, we designed three experiments during which participants were instructed to regulate negative and positive emotions while we systematically varied environmental factors and identified individual predispositions of the participants. In an effort to characterize emotion regulation deficits across a spectrum of relevant psychiatric disorders, we chose topics that are relevant for anxiety disorders, depression, and substance disorders:

- In study A, we confronted a sample of healthy participants with uncontrollable and unpredictable stress (environmental factor) and measured how participants' subclinical symptoms of anxiety and depression (individual factor) moderated their anxiety response.

- In study B, we administered gamma-hydroxybutyrate (GHB), a drug potentially used for depression in the future (environmental factor), to a sample of healthy participants and assessed how this influenced their sexual arousal, along with which neural underpinnings (individual factor) mediated this effect.

- In study C, we investigated in a clinical sample of cocaine users how chronic cocaine use (environmental factor) and three α_{2A}-adrenergic receptor polymorphisms (individual factor) mediated deficits in delaying gratification (coined as delay discounting).

Based on the diathesis-stress model, we hypothesized that specific environmental factors (like psychological stress, pharmacological stimulation, or chronic drug use) in combination with individual

predispositions (such as symptoms of anxiety and depression or genetic risk-alleles) lead to specifically measurable deficits in regulating negative (e.g., anxiety response) and positive emotions (e.g., sexual arousal) and in making decisions (e.g., delaying gratification).

1.3 Methods used to investigate emotion regulation deficits

To characterize emotion regulation deficits with different methods, we combined subjectively experienced emotions and behavioral measures with continuous skin conductance as a psycho-physiological measure in study A, functional magnetic resonance imaging (fMRI) as a neurobiological technique in study B, and genotyping single nucleotide polymorphisms as a genetic tool in study C.

Continuous skin conductance

Skin conductance (also known as electrodermal activity) refers to the phenomenon that arousing external or internal stimuli cause continuous variation in the electrical resistance properties of the skin (Boucsein, 2012). These resistance variations of the skin are based on the state of sweat glands controlled by the sympathetic nervous system (Carlson, 1994). Because of these properties, skin conductance can be used as an indicator of psychological and physiological arousal (i.e., emotional reactions).

In our project, continuous skin conductance was recorded using the BioPac system and MP30 Acquisition Box (BIOPAC Systems Inc., Goleta, CA, USA) with the corresponding isotonic gel electrodes (11mm contact area) placed on the palmar surfaces of the distal phalanx of the first and second digits of the left hand (Scerbo et al., 1992). Data pre-processing and analysis were performed with the MATLAB toolbox SCRalyze (http://scralyze.sourceforge.net) using a convolution model for how sudomotor bursting causes fluctuations in skin conductivity. This approach has been shown to be a better predictor of autonomic arousal than conventional measures (Bach et al., 2010).

Functional magnetic resonance imaging

Functional magnetic resonance imaging (fMRI) is an imaging technique visualizing brain activity by detecting associated changes in cerebral blood flow (Song et al., 2006). It is based on the assumption that the cerebral blood flow (the hemodynamic response) increases in brain regions that are activated because of associated increases in energy use (Song et al., 2006). The procedure uses the change in magnetization

between the oxygen-rich and oxygen-poor blood, the blood-oxygen-level dependent (BOLD) contrast, as an indicator for the cerebral blood flow and the associated brain activity (Song et al., 2006).

In our project, functional time series were acquired with a sensitivity-encoded single-shot echo-planar imaging sequence (SENSE-sshEPI)(Schmidt et al., 2005). The fMRI protocol used the following acquisition parameters: TE=35ms, TR=2500ms (=82°), FOV=24cm, acquisition matrix=80x80 interpolated to 128x128, voxel size=3x3x3mm, 40 contiguous axial slices (placed along the anterior-posterior commissure plane), and SENSE factor R=2.0. For structural reference, a three-dimensional T1-weighted anatomical scan with the following FFE sequence was obtained: TR/TE=9.3/4.6ms, flip angle=8°, 160 sagittal slices, FOV 240×240 mm, voxel size=1×1×1mm. fMRI data were analyzed using SPM8 (www.fil.ion.ucl.ac.uk/spm/). Motion artifacts were corrected by realignment to the mean image, mean-adjusted by proportional scaling, normalized according to the unified segmentation normalization approach (2x2x2mm), and spatially smoothed using a 6-mm full-with at half maximum Gaussian kernel.

Single nucleotide polymorphisms

A single nucleotide polymorphism (SNP) is a common mutation (i.e., present in >1% of a population) of a single nucleotide in the DNA sequence of a given gene (Barreiro et al., 2008). Such polymorphisms may affect a gene's protein expression and as a consequence, the gene's function. In that case, such a polymorphism may alter the function or the structure of brain circuits, and leads to individual differences in traits or behaviors (e.g., a vulnerability for disorders) (Canli et al., 2009).

In our project, DNA for the SNP genotyping was extracted either from EDTA anticoagulated blood samples or from immortalized lymphoblastoid cell cultures after transforming the lymphocytes with Epstein-Barr virus. The isolation of the DNA followed the QIAGEN protocol for the Blood & Cell Culture DNA Isolation Maxi Kit (QIAGEN, Hilden, Germany). For PCR, we added 5µl of buffer containing the Universal PCR MasterMix (No AmpErase UNG) and the SNP Genotyping Assay (both provided by Applied Biosystems, Foster City, CA, USA) to 12.5ng air-dried DNA. PCR was performed according to the SNP Genotyping protocol supplied by Applied Biosystems. Both alleles were scored in a single well by measuring the fluorescence at the end of the PCR using a Tecan Ultra 384 reader (Tecan, Crailsheim, Germany). Excitation- and emission-wavelengths were 485 and 535 nm for the FAM-labeled probes and 535 and 590 nm for the VIC-labeled probes, respectively.

1.4 Overview of the three studies and the additional publications

The PhD project led to the following three publications that are either in press or submitted at present:

- Study A: Havranek MM, Bolliger B, Roos S, Pryce CR, Quednow BB, Seifritz E. Uncontrollable and unpredictable stress interacts with subclinical depression and anxiety scores in determining anxiety response. Stress. In press.
- Study B: Bosch OG*, Havranek MM*, Baumberger A, Preller KH, VonRotz R, Herdener M, Kraehenmann R, Stämpfli P, Scheidegger M, Seifritz E, Quednow BB. Neural underpinnings of prosexual effects induced by gamma-hydroxybutyrate in healthy humans. In submission. * contributed equally to this publication
- Study C: Havranek MM, Hulka LM, Tasiudi E, Eisenegger C, Vonmoos M, Preller KH, Mössner R, Baumgartner MR, Seifritz E, Grünblatt E, Quednow BB. α_{2A}-adrenergic receptor polymorphisms and mRNA expression levels are associated with delay discounting in cocaine users. Addict Biol. In press.

In addition to the three main studies, two further publications arose from this PhD project. They are different from the three main studies in terms of content but for the sake of completeness, they will be listed here as well:

- Havranek MM, Volkart F, Bolliger B, Roos S, Buschner M, Mansour R, Chmielewski T, Gaudlitz K, Hättenschwiler J, Seifritz E, Ruch W. The fear of being laughed at as a diagnostic criterion in social anxiety disorder and avoidant personality disorder. In submission.
- Havranek MM, Vonmoos M, Müller CP, Büetiger JR, Tasiudi E, Hulka LM, Preller KH, Mössner R, Grünblatt E, Seifritz E, Quednow BB. Serotonin transporter and tryptophan hydroxylase gene variations mediate working memory deficits of cocaine users. Neuropsychopharmacology. 2015. doi: 10.1038/npp.2015.146.

Summaries of the main studies

In study A, we investigated the impact of uncontrollable and unpredictable stress on the anxiety response of healthy participants with varying subclinical depression and anxiety scores. Uncontrollable stress is assumed to be a critical etiological factor in the pathogenesis of depression. In contrast, unpredictability of stressors is assumed to facilitate the development of sustained anxiety. Despite the frequent co-morbidity of depression and anxiety disorders, these two factors have rarely been studied simultaneously

in humans. Therefore, we investigated whether there are interaction effects of uncontrollability and unpredictability on anxiety response in healthy participants. 79 healthy participants performed a visual dot probe task with emotional faces while receiving mild electrical shocks in four different conditions (2x2 factorial design). In (un)controllable conditions, participants were (not) able to attenuate shock intensity. In (un)predictable conditions, participants were (not) able to anticipate shock occurrence. Before the experiment, participants' subclinical depression and anxiety scores were measured using the Beck Depression and Anxiety Inventories (BDI/BAI). During the experiment, continuous skin conductance and self-reported state anxiety were assessed and attentional biases towards angry faces were calculated. As expected, participants showed greater anxiety in uncontrollable compared to controllable conditions, and in unpredictable compared to predictable conditions. Additionally, anxiety decreased within the test sessions in participants with low BDI/BAI scores but not in participants with higher BDI/BAI scores. Most importantly, controllability and predictability interacted with each other and with BDI/BAI scores with regard to anxiety. Our results provide evidence that uncontrollability and unpredictability of stressors have not only separate effects, but also interaction effects on several anxiety measures in susceptible individuals. The relevance of this finding concerning emotion regulation will be detailed in the overall discussion at the end of this PhD project.

In study B, we examined the effects of GHB on sexual arousal, along with which neural underpinnings mediated this reponse. *GHB* is a GHB-/GABAB-receptor agonist used as treatment for narcolepsy but also as a drug of abuse. Non-medical users repeatedly report prosexual properties, including lowering of sexual standards for partner selection. Given that sexual dysfunction is a critical illness- and treatment-related impairment often occurring in psychiatric conditions such as depression, we aimed to investigate the neural mechanism of GHB-induced sexual arousal. We characterized putative prosexual effects of GHB in healthy males in two experiments, both employing a randomized, placebo-controlled, double-blind, balanced, and cross-over design. In experiment I, the subjective and behavioral effects of 25 and 35 mg/kg GHB (p.o.) vs. placebo were tested in 32 participants using the Sexual Arousal and Desire Inventory and the Sexual Arousal Task. In experiment II, the brain reactivity towards erotic vs. neutral pictures was investigated in 19 participants with fMRI after 35 mg/kg GHB (p.o.). Experiment I revealed acute prosexual effects of GHB in the subjective measures, but not in the behavioral measures, while experiment II showed that the erotic stimuli activated the anterior cingulate cortex, precentral and postcentral gyri, inferior parietal lobule, and the thalamus in the placebo condition. After GHB administration, these areas were equally activated already by neutral pictures, and subjective sexual arousal was enhanced. In contrast, GHB compared to placebo decreased neural response to erotic pictures, although subjective sexual arousal

was at its highest. In conclusion, GHB stimulates hedonic sexual functioning and lowers the threshold for erotic perception. The importance of this finding concerning emotion regulation will be detailed in the overall discussion at the end.

In study C, we investigated how chronic cocaine use influenced the ability to delay gratification, and how this effect was mediated by three α_{2A}-adrenergic receptor polymorphisms. Cocaine users characteristically display preferences for smaller immediate rewards over larger delayed rewards, and this delay discounting (DD) has been proposed as an endophenotype of cocaine addiction. Recent evidence suggests that the norepinephrine system, and more specifically the α_{2A}-adrenergic receptor (*ADRA2A*), are impacted by chronic cocaine use, while also being potentially involved in the neural mechanisms underlying DD. Hence, we investigated the effects of *ADRA2A* polymorphisms and *ADRA2A* mRNA expression levels on DD of cocaine users and stimulant-naïve controls. 223 participants (129 cocaine users, 94 stimulant-naïve healthy controls) completed a computerized DD paradigm and were genotyped for three single nucleotide polymorphisms (SNPs; rs1800544, rs521674, and rs602618) in the *ADRA2A* gene, whilst their peripheral *ADRA2A* mRNA expression was quantified in whole blood samples. The three SNPs were in nearly perfect linkage disequilibrium. Accordingly, significant group*genotype interactions were found for all three *ADRA2A* variants, revealing steeper DD in cocaine users (but not in controls) carrying the G-allele of SNP rs1800544, the T-allele of rs521674, and the C-allele of rs602618. Similarly, high *ADRA2A* mRNA expression levels were significantly associated with a reduced tendency to choose smaller, more immediate rewards (over larger delayed rewards) in cocaine users, but not in controls. As the relationship between DD and cocaine use was moderated by *ADRA2A* SNPs and by peripheral *ADRA2A* gene expression, we propose that the norepinephrine system is involved in DD deficits observed in cocaine using individuals. Again, the relevance of this finding concerning emotion regulation will be detailed in the overall discussion.

2 Study A: Uncontrollable and unpredictable stress interacts with depression and anxiety symptoms in determining anxiety response

This chapter is an adapted version of the paper: Havranek MM, Bolliger B, Roos S, Pryce CR, Quednow BB, Seifritz E. Uncontrollable and unpredictable stress interacts with subclinical depression and anxiety scores in determining anxiety response. Stress. In press. Contributions of Michael Havranek: everything from designing the experiment to writing the publication. KEK-ZH-Nr. 2011-0338.

2.1 Introduction

Depression and anxiety disorders are among the most common and costly diseases in the world (Beddington et al., 2008, Greenberg et al., 2003, Kessler et al., 2005). They occur co-morbidly strikingly often with more than 50% of depressed patients reporting a lifetime history of anxiety disorders (Kaufman & Charney, 2000, Kessler et al., 2005). The presence of a co-morbid pathology substantially increases life impairment of patients and is associated with worse treatment outcome and increased likelihood of chronicity (Hirschfeld, 2001). However, there is still limited understanding of the psychological mechanisms underlying these disorders and their frequent co-morbidity.

Stress is the main known etiological factor associated with the pathogenesis of affective disorders (Kendler et al., 1999). However, the nature of the stress experience determines its impact on psychological health. For example, behavioral or cognitive control over stressors (referred to as *controllability*) and predictability of stressors have been suggested to moderate the stress experience and its negative influences on health (Hammen, 2005). The effects of controllability and predictability have initially been investigated simultaneously in animals (Mineka & Kihlstrom, 1978, Seligman, 1975, Thomas & Dewald, 1977, Wolpe, 1958). However, in accordance with the suggestion that experiencing uncontrollability generally leads to depression, whereas experiencing unpredictability generally leads to anxiety (Seligman, 1975), subsequent research mainly investigated either the individual effects of uncontrollability or the individual effects of unpredictability separately (Zvolensky et al., 2000).

Encouraged by the popularity of the *Learned Helplessness Model* (Maier & Seligman, 1976), many researchers focused on the impact of uncontrollable stress in the development of depression (see e.g., Pryce et al., 2011 for a review of animal and human evidence). Seligman and Maier (1976) demonstrated that animals confronted with uncontrollable stress developed escaping deficits whereas those confronted with the same amount of controllable stress did not. Experiments in healthy humans replicated these

findings by demonstrating feelings of helplessness, behavioral changes (error rates and reaction times) and slow cortical potential changes during and after exposure to uncontrollable compared to controllable stress (Diener et al., 2009). Additionally and importantly, this influence of uncontrollability was more pronounced in depressed patients compared to healthy controls (Diener et al., 2009). Furthermore, other studies demonstrated that the perception of controllability not only prevents immediate negative effects of stress and immunizes against future situations of uncontrollable stress (Diener et al., 2009), but also reduces anxiety regarding future aversive events (such as pain stimuli, see e.g., Salomons et al., 2004, Wiech et al., 2006).

Concerning predictability, the group of Grillon investigated the effects of unpredictable stressors on sustained contextual anxiety. They showed in several experiments that unpredictable aversive events increased sustained anxiety, whereas predictable events only increased phasic cued fear of the specific signaling cue (Grillon et al., 2004, Mol et al., 2007). They posited the *Safety Signal Hypothesis* (Grillon, 2002) as a potential explanation for this finding: if an aversive event is signaled by a cue, the absence of that cue signals the absence of danger or the presence of safety. However, if the aversive event is not signaled by a cue and is thus unpredictable, there are no periods of safety and the individual remains in a state of sustained anxious anticipation. Furthermore, they demonstrated that anxiety of unpredictable but not predictable aversive events was increased in high anxious individuals and in patients with panic disorders and posttraumatic stress disorder (Glotzbach-Schoon et al., 2013, Grillon et al., 2008, 2009).

Experiments in rats have provided strong evidence that the stress buffering effects of controllability and predictability are mediated by two different neural pathways (Christianson et al., 2008). It is assumed that when stressors are controllable, the stress-induced activation of the dorsal raphe nucleus is inhibited by the ventromedial prefrontal cortex (Amat et al., 2005). On the other hand, the anxiety-preventing effects of predictability seem to be mediated by the posterior insula, which in turn inhibits the amygdala (Christianson et al., 2008). These neural underpinnings indicate that uncontrollability and unpredictability may not only have separate effects but have the neurobiological potential for interaction effects via connection between the amygdala and the dorsal raphe nucleus (Peyron et al., 1998). Considering the susceptibility of depressed patients to uncontrollability (Diener et al., 2009) and the susceptibility of anxiety patients to unpredictability (Grillon et al., 2008, Grillon et al., 2009), we hypothesized that the co-occurrence of experimental stressors that are both uncontrollable and unpredictable would act additively or even synergistically on anxiety response in participants.

Despite the considerable scientific interest in the concepts of controllability and predictability, only a few studies have examined them simultaneously in humans. On the contrary, most studies did not differentiate

between them or even confounded them (Miller, 1979, Zvolensky et al., 2000). One study that did investigate both factors simultaneously in a sample of healthy participants found separate effects on heart rate reactivity but no interaction effects (Baker & Stephenson, 2000). Because of this, we hypothesized that interaction effects may be restricted to participants with subclinical symptoms of depression or anxiety disorders. Thus, we developed a paradigm to simultaneously examine the effects of the uncontrollability and unpredictability of a stressor (i.e., mild electrical shocks) on multiple anxiety measures (skin conductance, self-reported anxiety, and attentional bias towards threat) and the Positive and Negative Affect Schedule (PANAS) as a state measure of mood (Watson et al., 1988) in a sample of healthy participants. Notably, our sample showed varying levels of subclinical depression and anxiety scores as assessed by the Beck Depression Inventory (BDI, Beck et al., 1961) and the Beck Anxiety Inventory (BAI, Beck et al., 1988). We tested the following three hypotheses: (1) separate main effects of stressor uncontrollability and unpredictability can be found on the anxiety measures, (2) participants with elevated depression scores are particularly susceptible to uncontrollability, whereas participants with more pronounced anxiety scores are particularly susceptible to unpredictability, and (3) under inclusion of the depression and anxiety scores of the participants, interaction effects of uncontrollability and unpredictability will be found.

2.2 Methods

Participants

Seventy-nine participants (42 males and 37 females; aged 18 - 37 years, 24.15 $\pm$ 4.75 [*SD*]) took part in the experiment. They were recruited via online advertisements on the University of Zurich website. All participants reported no past or present neurological or psychiatric diseases requiring treatment and no sensory impairments or subjective cognitive impairments. Additional exclusion criteria were regular illegal drug use, regular use of prescription drugs, and left-handedness. All participants were instructed to abstain from drinking alcohol for 24 hours before the experiment. The presented study was approved by the Ethics Committee of the Canton Zurich. All participants gave written informed consent prior to the study and received a moderate financial compensation for participation.

Task

Individuals had to perform a visual dot probe task (Bradley et al., 1999, Koster et al., 2004, Mogg et al., 1997) while being under threat of receiving multiple mild electrical shocks. On a computer screen (placed 50cm in front of them), a fixation cross (1000ms), two faces (50ms), and a dot appearing in the former place of one of the two faces (1000ms), were presented consecutively. Intertrial intervals varied randomly between 750ms and 1000ms (as described previously: Herry et al., 2007). Participants were informed that they had to respond to the position of the dot by pressing a keyboard button (right or left). Face pictures depicted either pairs of two neutral faces or pairs of a neutral and an angry face, which were chosen from the Ekmann set (Ekman & Friesen, 1975) and were previously selected and morphed to optimize affect recognition performance in a normative sample (Schmidt-Daffy, 2011). Using a within-subject design, the participants had to perform all four experimental conditions (see below) whereby each condition consisted of 80 trials (40 neutral-neutral pairings, 40 neutral-angry pairings, see below) and an approximate length of 3 minutes (depending on the average reaction time of each individual).

Stressor controllability and predictability

During each of the four conditions, each participant received exactly eight mild electrical shocks (300volt, 1000ms, either 19 or 26mA, see below) applied to the back of their right hand via foam electrodes with conductive adhesive hydrogel using a commercially available electric stimulation device (Constant Current Stimulator, model DS7A; Digitimer, Hertfordshire, UK). A 2x2 factorial design was used to implement stressor controllability and predictability during the four conditions of the visual dot probe task: *controllable-predictable* (C+P+), *controllable-unpredictable* (C+P-), *uncontrollable-predictable* (C-P+) and *uncontrollable-unpredictable* (C-P-). Individuals were informed that under controllable conditions (C+P+, C+P), shocks were attenuated if they responded fast enough to the position of the dot, whereas in uncontrollable conditions their response speed would have no effect on shock intensity. In fact, based on previous pilot studies, shock intensity was presented in both conditions (19mA in controllable and 26mA in uncontrollable conditions) independent of subject's response speed, to ensure the experience of control in all participants. Furthermore, participants were told that in predictable conditions (C+P+, C-P+), shocks would only occur after a danger cue and never after a safety cue, whereas in unpredictable conditions, shocks would occur independently of cue. The danger and safety cues were implemented using two different shapes of the fixation cross. Specifically, the fixation cross was either presented as a straight ("+") or oblique ("x") cross. Hereby, in predictable conditions, the respective cue signaling danger appeared in

20 trials, whereas the cue signaling safety appeared in 60 trials. Individuals were informed before each condition which cue signaled danger. In unpredictable conditions, the occurrence probability (20 trials vs. 60 trials) of the two forms ("+" vs. "x") was similar; however, they were unrelated to shock occurrence. Cues were counterbalanced across participants and semi-randomized across conditions. The order of the four conditions was counter-balanced across participants.

Anxiety measures

Skin conductance: Continuous skin conductance (SC) was recorded during task performance as a measure of physiological arousal using the BioPac system and MP30 Acquisition Box (BIOPAC Systems Inc., Goleta, CA, USA) with the corresponding isotonic gel electrodes (11mm contact area) placed on the palmar surfaces of the distal phalanx of the first and second digits of the left hand (Scerbo et al., 1992). Data pre-processing and analysis were performed with the MATLAB toolbox *SCRalyze* (http://scralyze.sourceforge.net) using a convolution model for how sudomotor bursting causes fluctuations in skin conductivity. This approach has been shown to be a better predictor of autonomic arousal than conventional measures (Bach et al., 2010).

Self-reported anxiety: Self-reported anxiety was assessed as a measure of subjective anxiety twice during each experimental condition (once after approximately 20 trials and once after approximately 60 trials) on a visual analogue scale ranging from 1 to 10 (1 = "I am not feeling anxious/nervous at all", 10 = "I am feeling extremely anxious/nervous").

Attentional bias: Attentional bias towards angry faces was used as a measure of selective attention towards threat (Bradley et al., 1999, Koster et al., 2004, Mogg et al., 1997). To calculate the attentional bias, trials with neutral-angry pairings were divided into *congruent trials* (in which the dot appeared in the former position of the angry face) and *incongruent trials* (in which the dot appeared in the former position of the neutral face). Then, mean reaction times in congruent trials were subtracted from mean reaction times in incongruent trials for each individual. It must be noted that we focused on attentional biases from non-danger trials for further analyses to solely compare effects of sustained anxiety without confounds of phasic cued fear from the predictable conditions.

Procedure

Individuals were introduced to the experiment and were asked to fill out the BDI (Beck et al., 1961) and the BAI (Beck et al., 1988). Then, SC and electrical stimulation electrodes were attached and participants were presented with written instructions for the visual dot probe task on the computer screen followed by a first practice trial. During the experiment they were explicitly informed before each condition which condition would follow and had to go through a short practice trial tailored for this specific condition. After every condition, we additionally assessed a state measure of participants' mood using the PANAS (Watson et al., 1988). However, we did not find any significant results and because of this the PANAS data are not presented here. Following completion of the experiment, participants were debriefed and asked about whether they have experienced control over stressors in the controllable conditions.

Statistical analyses

Effects of stressor controllability and predictability were analyzed separately for each anxiety measure using analyses of variance (ANOVA) with the factors: *controllability* (controllable vs. uncontrollable) and *predictability* (predictable vs. unpredictable). Influences of BDI and BAI scores were subsequently analyzed using analyses of covariance (ANCOVA) with continuous *BDI* and *BAI scores as* covariates. To illustrate the hereby discovered interactions, we used a 2x2x2x2 ANOVA with the factors *controllability*, *predictability*, *BDI* (low vs. high BDI scores, using the median split=4), and *BAI* (low vs. high BAI scores, using the median split=8). For self-reported anxiety, we additionally included the factor *time* (anxiety assessed after 20 trials vs. after 60 trials during each condition) into our analyses. Finally, *post hoc t*-tests corrected for multiple comparisons (Bonferroni) were conducted on the basis of significant ANOVA effects. Statistical analyses were conducted with SPSS (Version 20.0) and results were considered significant if $p<0.05$ after correction for multiple comparisons.

2.3 Results

Manipulation check and group stratification

In the manipulation check of controllability after the experiment, 97.5% of all participants reported to have experienced behavioral control (of attenuating the shocks themselves). As a manipulation check of predictability, reaction times (RT) were shorter in trials following danger cues compared to trials following

no-danger cues in predictable but not in unpredictable conditions. This indicates increased response speed of participants when anticipating shocks (see Table 1).

Table 1. Reaction times (RT, ms) of danger trials and no-danger trials (means and *SD*)

	RT danger trials mean (*SD*)	**RT no-danger trials mean (*SD*)**	***t (df)***	***p***
C+P+	354.5 (59.8)	360.4 (59.8)	-2.8 (78)	**0.007**
C-P+	357.0 (54.1)	365.9 (50.4)	-3.1 (78)	**0.004**
C+P-	352.8 (42.0)	347.5 (39.8)	3.0 (78)	**0.003**
C-P-	364.0 (51.9)	360.6 (51.7)	1.6 (78)	0.113

BDI and BAI scores were significantly correlated ($r(79)=-0.33$, $p=.003$). BDI scores ranged from 0 to 30 (4.9 $\pm$4.96 [*SD*]) and BAI scores from 0 to 39 (11.38 $\pm$10.34). Within both stratified groups (low vs. high BDI and low vs. high BAI scores), participants did not differ regarding age, sex distribution or education (see Table 2). They also did not differ regarding average RT or average anxiety scores in all three measures (SC, self-reported anxiety, attentional bias) except for a difference in SC between participants with low and high BDI scores (see Table 2).

Table 2. Demographic data and average anxiety scores for participants with low vs. high BDI and BAI scores (means and *SD* / frequency data)

	Low BDI (*n* = 45) mean (*SD*) / *N*	**High BDI (*n* = 34) mean (*SD*) / *N***	***t (df) / X^2(df)***	***p***
Age	24.0 (4.9)	24.3 (4.6)	-0.3 (77)	0.792
Sex (male / female)	26 / 19	16 / 18	0.9 (1)	0.371
Education (secondary / higher)	7 / 38	3 / 31	0.8 (1)	0.502
Average reaction time (ms)	360.0 (54.0)	355.8 (32.1)	0.4 (77)	0.692
Average skin conductance (µmho)	0.9 (0.6)	0.6 (0.5)	2.5 (77)	**0.013**
Average self-reported anxiety (points)	4.2 (1.8)	3.9 (1.3)	1.0 (77)	0.339
Average attentional bias (ms)	-1.6 (9.4)	0.1 (11.2)	-0.8 (77)	0.448
	Low BAI (*n*=37) mean (*SD*) / *N*	**High BAI (*n*=42) mean (*SD*) / *N***	***t (df) / X^2(df)***	***p***
Age	24.6 (5.2)	23.8 (4.3)	0.8 (77)	0.440
Sex (male / female)	20 / 17	22 / 20	0.0 (1)	0.999
Education (secondary / higher)	5 / 32	5 / 37	0.0 (1)	0.999
Average reaction time (ms)	366.3 (56.1)	351.0 (33.2)	1.5 (77)	0.139
Average skin conductance (µmho)	0.7 (0.5)	0.9 (0.6)	-1.2 (77)	0.241
Average self-reported anxiety (points)	3.8 (1.6)	4.3 (1.7)	-1.6 (77)	0.115
Average attentional bias (ms)	-1.4 (10.0)	-0.4 (10.4)	-4.3 (77)	0.671

BAI = Beck Anxiety Inventory (BAI) median split, BDI = Beck Depression Inventory (BDI) median split

Separate effects of controllability and predictability

Investigating the influence of stressor controllability and predictability on anxiety, we found significant main effects for both *controllability* ($F(1,78)=10.38$, $p<.01$) and *predictability* ($F(1,78)=6.00$, $p<.05$) in SC, a significant effect for *controllability* in self-reported anxiety ($F(1,78)=23.14$, $p<.001$) and an almost significant effect for *predictability* in attentional bias ($F(1,78)=3.77$, $p=.056$). However, there was no significant interaction effect of *controllability by predictability* in any measure. The main effects of *controllability* and *predictability* indicated higher SCs (see Figure 1A) and higher self-reported anxiety (see Figure 1B) in conditions with uncontrollable shocks (compared to conditions with controllable shocks) and higher SCs (see Figure 1A) and higher attentional biases (see Figure 1C) in conditions with unpredictable shocks (compared to conditions with predictable shocks). Furthermore, comparing SC in the four individual conditions revealed that C-P- elicited highest SC, followed by C-P+, which in turn was followed by C+P- and lowest SC was elicited in C+P+ (see Figure 1A).

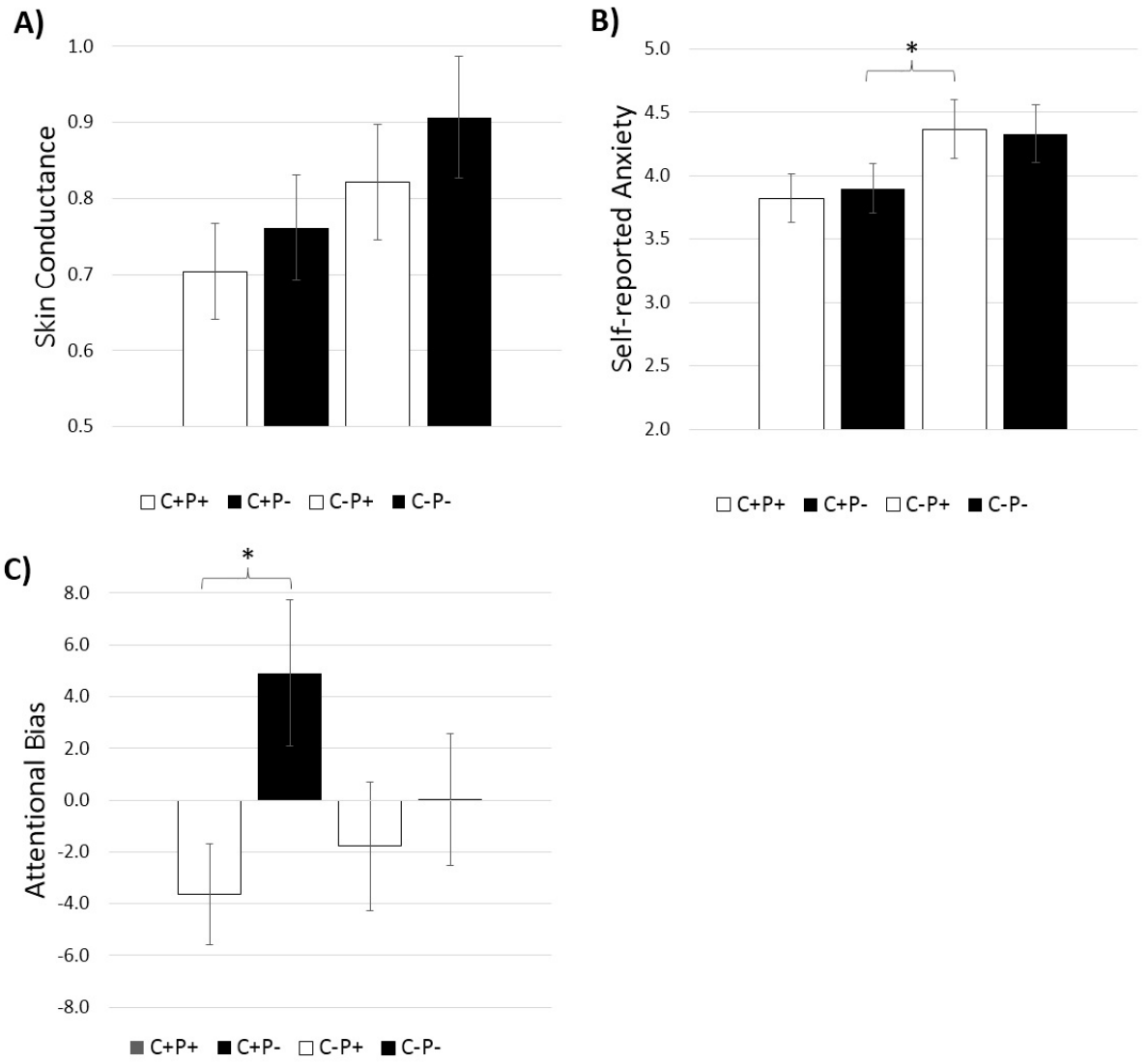


Figure 1. Depicted are mean and *standard errors of mean* for skin conductance (µmho) (Fig. 1A), self-reported anxiety (points) (Fig. 1B) and attentional bias (ms) (Fig. 1C) in the four conditions: controllable-predictable (C+P+), controllable-unpredictable (C+P-), uncontrollable-predictable (C-P+) and uncontrollable-unpredictable (C-P-). Significance levels: *p<.05, **p<.01, ***p<.001 (Bonferroni corrected for multiple comparisons).

Interactions of controllability and predictability with subclinical scores

Introducing BDI and BAI scores as continuous covariates in our calculations revealed significant interaction effects in SC and self-reported anxiety. Beyond the remaining significant main effects of *controllability* and *predictability* in all measures, we observed interactions of *predictability by BAI scores* in SC (F(1,76)=4.07,

p<.05) and of *controllability by BDI scores by time* (*F*(1,76)=5.63, *p*<.05) and *predictability by BAI scores by time* (*F*(1,76)=4.87, *p*<.05) in self-reported anxiety.

To illustrate the discovered interactions, we then stratified participants into groups with low vs. high BDI scores and with low vs. high BAI scores. As above, we found significant main effects of *controllability* and *predictability* in SC, a significant main effect of *controllability* in self-reported anxiety and a significant main effect for *predictability* in attentional bias (see Table 3). Additionally, we observed a significant threefold interaction of *controllability by predictability by BDI* in SC, two significant interactions of *controllability by BDI by time* and of *predictability by BAI by time* in self-reported anxiety, and an interaction of *controllability by predictability by BAI* in attentional bias (see Table 3).

Table 3. Analysis of variance for skin conductance, self-reported anxiety and attentional bias

ANOVA results	**Skin Conductance**				**Self-Reported Anxiety**				**Attentional Bias**			
	F	*df*	*p*	ηp^2	*F*	*df*	*p*	ηp^2	*F*	*df*	*p*	ηp^2
Main effects												
Control	10.80	75	**.002**	.126	22.22	75	**.000**	.229	0.83	75	.338	.012
Control*BAI	0.28	75	.596	.004	0.05	75	.828	.001	1.05	75	.307	.014
Control*BDI	0.73	75	.397	.010	0.27	75	.604	.004	1.49	75	.226	.019
Prediction	4.66	75	**.034**	.058	0.08	75	.777	.001	4.64	75	**.034**	.058
Prediction*BAI	0.09	75	.764	.001	0.00	75	.951	.000	0.00	75	.995	.000
Prediction*BDI	0.13	75	.716	.002	0.18	75	.676	.002	1.78	75	.186	.023
Control*Prediction	0.14	75	.710	.002	0.33	75	.569	.004	3.21	75	.077	.041
Control*Prediction*BAI	0.37	75	.545	.005	0.13	75	.715	.002	5.45	75	**.022**	.068
Control*Prediction*BDI	6.02	75	**.016**	.074	1.30	75	.258	.017	0.10	75	.749	.001
Time effects												
Time					1.51	75	.223	.020				
Time*BAI					2.15	75	.147	.028				
Time*BDI					0.26	75	.612	.003				
Control*Time					3.46	75	.067	.044				
Control*Time*BAI					0.18	75	.676	.002				
Control*Time*BDI					7.42	75	**.008**	.090				
Prediction*Time					0.51	75	.477	.007				
Prediction*Time*BAI					5.64	75	**.020**	.070				
Prediction*Time*BDI					1.15	75	.288	.015				

BAI = Beck Anxiety Inventory (BAI) median split, BDI = Beck Depression Inventory (BDI) median split

The interaction for SC revealed that in participants with low BDI scores, shock unpredictability further increased SC in the uncontrollable condition, whereas in the controllable condition, shock unpredictability did not affect SC. In participants with high BDI scores, unpredictability did not increase SC in the uncontrollable condition, while it did additionally increase it in the controllable condition (see Figure 2A).

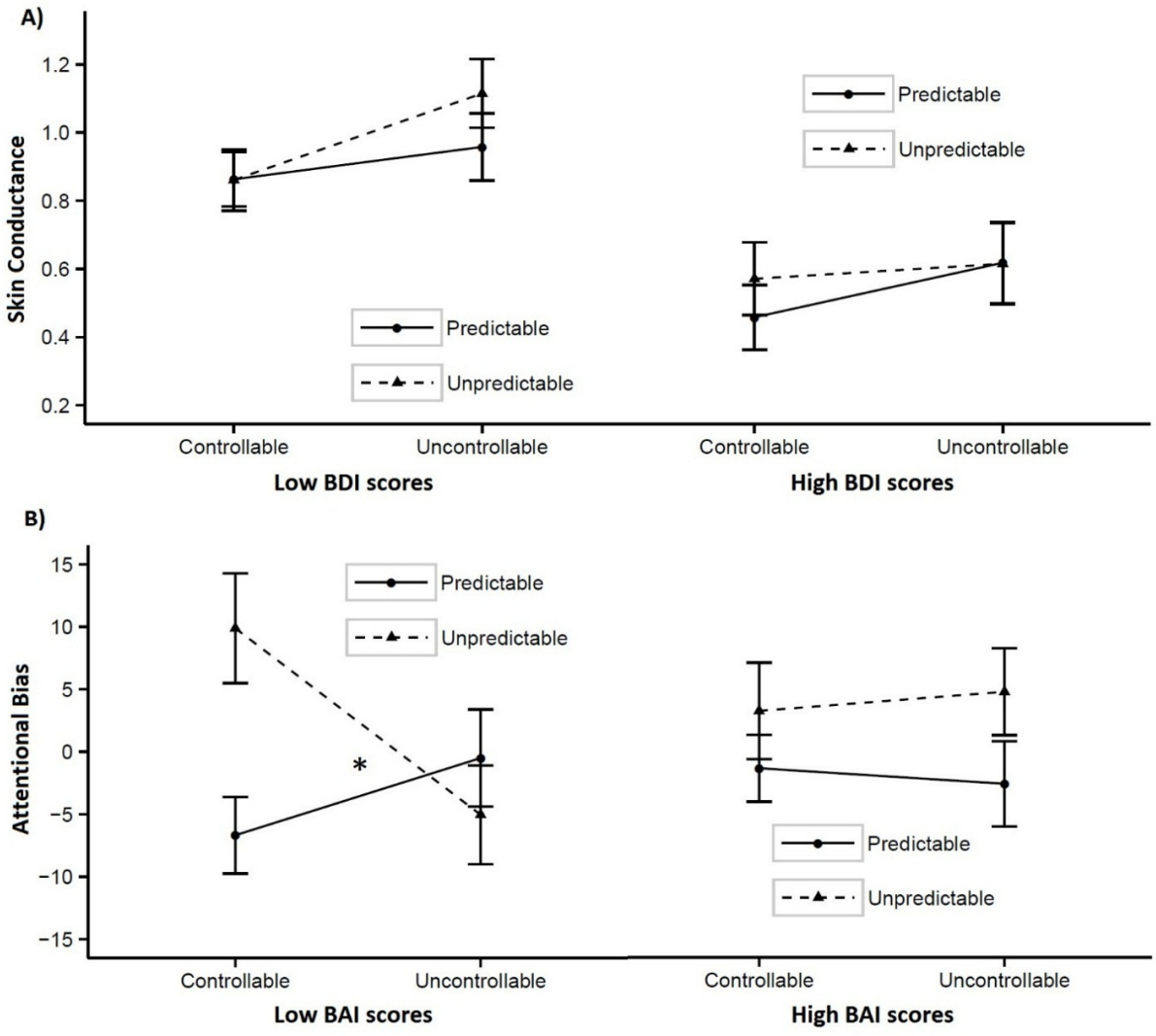


Figure 2. Significant threefold interactions in skin conductance (µmho) for participants with low Beck Depression Inventory (BDI) scores and participants with high BDI scores (Fig. 2A) and in attentional bias (ms) for participants with low Beck Anxiety Inventory (BAI) scores and participants with high BAI scores (Fig. 2B). An additionally significant twofold interaction in attentional bias is indicated with * (corrected for multiple comparisons).

The interaction of *controllability by BDI by time* indicated that in participants with low BDI scores the self-reported anxiety in both controllable and uncontrollable conditions decreased within test sessions, whereas in participants with higher BDI scores the self-reported anxiety in uncontrollable conditions increased within test sessions (see Figure 3A).

The interaction of *predictability by BAI by time* revealed that in participants with low BAI scores the self-reported anxiety in both unpredictable and predictable conditions decreased within test sessions, while in participants with higher BAI scores the self-reported anxiety in unpredictable and predictable conditions did not decrease; on the contrary, the self-reported anxiety in the predictable conditions even showed a trend towards an increase in participants with higher BAI scores (see Figure 3B).

Finally, the interaction in attentional bias reflected that in participants with low BAI scores, only the uncontrollable-predictable condition (C-P+) elicited a positive attentional bias, while in participants with higher BAI scores, both unpredictable conditions elicited a positive attentional bias (see Figure 2B).

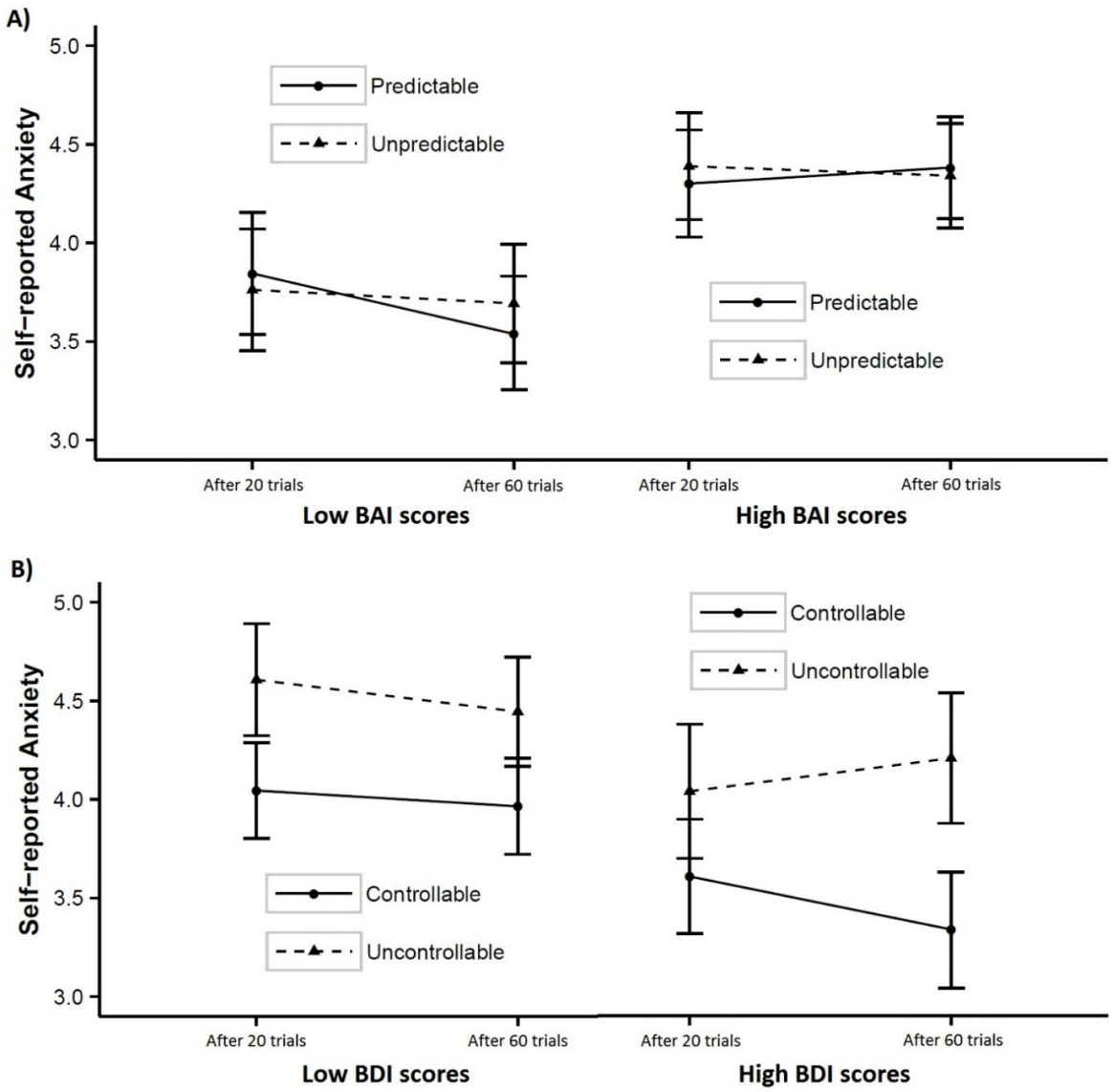


Figure 3. Significant threefold interactions in self-reported anxiety (points) within test session (measured after 20 trials and after 60 trials) for participants with low Beck Anxiety Inventory (BAI) scores and participants with high BAI scores (Fig. 3A) and for participants with low Beck Depression Inventory (BDI) scores and participants with high BDI scores (Fig. 3B).

2.4 Discussion

Studies simultaneously investigating controllability and predictability are rare in humans. Previous research mainly focused either on uncontrollability inducing feelings of helplessness and depression, or on unpredictability eliciting sustained anxiety. The few existing studies investigating the two factors simultaneously did not yield interaction effects (Baker & Stephenson, 2000). We hypothesized that they failed to find interactions because they did not factor in susceptibilities of participants. Therefore, we designed a paradigm enabling the distinct but simultaneous examination of the effects of uncontrollable and unpredictable stress (electrical shocks) on multiple anxiety measures (skin conductance, self-reported anxiety and attentional bias towards threat) in healthy participants stratified according to subclinical symptoms of depression (BDI) and anxiety (BAI). With this approach, we revealed not only separate main effects of *controllability* and *predictability*, but also interaction effects of the two factors with subclinical scores of depression and anxiety in all three anxiety measures. These findings may provide insights into the psychological mechanisms underlying a depressive/anxiety co-morbidity.

Concerning the separate main effects of *controllability* and *predictability*, we were able to replicate previous findings. Here, we demonstrate that conditions with uncontrollable shocks elicited increased physiological arousal and increased self-reported anxiety compared to conditions with controllable shocks. These findings are consistent with animal studies on learned helplessness (Azzinnari et al., 2014) reporting escape-deficits together with increased anxiety of anticipated shocks when mice were administered uncontrollable stressors (compared to controllable stressors). Furthermore, previous studies in humans (Baker & Stephenson, 2000, Breier et al., 1987, Peters et al., 1998) also demonstrated task performance reductions and increases in heart rate reactivity when participants experienced uncontrollable stress experiences. Regarding predictability, we found increased physiological arousal as well as higher attentional biases towards threat in conditions with unpredictable shocks in contrast to conditions with predictable shocks. These findings are in line with multiple studies from the group of Grillon et al. (see e.g., Grillon et al., 2004, Mol et al., 2007) demonstrating that healthy participants revealed increased startle reflexes as an index of increased sustained anxiety in situations with unpredictable compared to predictable stressors. In addition, this is also consistent with translational evidence showing that even unpredictabiliy per se (without any threat) induces anxiety-like behavioral and neural effects in humans and mice (Herry et al., 2007).

Previous research has demonstrated that depressed participants, compared to healthy controls, felt more helpless and showed more altered information processing when confronted with uncontrollability (Diener et al., 2009). Similarly, high anxious individuals as well as patients with panic disorders and post-traumatic

stress disorders showed elevated startle magnitudes in unpredictable stressor situations compared to healthy controls (Glotzbach-Schoon et al., 2013, Grillon et al., 2008, 2009). Consistent with these studies, we found interactions of depression traits (BDI) with *controllability* and interactions of anxiety traits (BAI) with *predictability* in self-reported state anxiety. More specifically, we demonstrated that in participants with low BDI scores, the self-reported anxiety in both controllable and uncontrollable conditions decreased within test sessions, while the self-reported anxiety in the uncontrollable conditions increased within test sessions in participants with higher BDI scores. This finding is plausible if we base its interpretation on the learned helplessness model (Seligman, 1975). Accordingly, participants with low BDI scores might have more frequently experienced control over their life circumstances which immunized them against the effects of the experimentally implemented uncontrollability. This interpretation is supported by findings demonstrating that after experiencing a loss of control, depressed patients (in contrast to healthy controls) continued to feel helpless after control had been reinstated (Diener et al., 2009). Regarding predictability, we found that in participants with low BAI scores, self-reported anxiety in both the predictable and the unpredictable conditions decreased within test sessions, whereas this decrease of anxiety was not seen in participants with higher BAI scores. On the contrary, self-reported anxiety in the predictable conditions slightly increased within test sessions in high anxious individuals. These results are supported by research on fear and anxiety learning which demonstrates that in high anxious individuals both fear and anxiety associations are acquired faster and remain more stable over time than in healthy controls (Barrett & Armony, 2009, Sehlmeyer et al., 2011).

Stratifying our participants into groups with low vs. high depression and low vs. high anxiety scores revealed significant interaction effects of *controllability, predictability* and BDI for SC and of *controllability*, *predictability* and *BAI* for attentional bias. More precisely, in participants with low BDI scores, unpredictability further increased physiological arousal only in the uncontrollable but not in the controllable condition. In participants with high BDI scores, on the other hand, unpredictability did increase arousal in the controllable condition, while it did not additionally affect it in the uncontrollable condition. It must be highlighted that the lower overall physiological arousal level in participants with higher BDI scores compared to participants with low BDI scores (see Figure 2A and Table 2) seems to be a characteristic finding in depressed individuals which has been reported on multiple occasions (Allen et al., 1999, Mardaga & Hansenne, 2009, McTeague et al., 2009, Thorell, 2009). Because of this, we restricted the interpretation of this finding to the relative effects of uncontrollability and unpredictability. There is, to the best of our knowledge, no previous research on the interaction of control and prediction with (sub-)clinical symptoms but our findings can be interpreted in light of the learned helplessness theory again.

Assuming that participants with higher BDI scores have previously experienced helplessness, exposure to unpredictable shocks might be sufficient to induce a feeling of helplessness leading to heightened arousal even if shocks are controllable (in C+P-) (see also: Diener et al., 2009). In contrast, participants with low BDI scores might have low susceptibility to helplessness, and unpredictability of shocks only increased arousal when shocks actually were uncontrollable (in C-P-). Based on these results, we believe that previous studies did not find interaction effects of controllability and predictability because they did not take participants' depression or anxiety trait scores into account.

Moreover, we demonstrate that both participants with low and participants with higher anxiety (BAI) scores showed negative attentional biases in the predictable conditions. However, in participants with higher BAI scores both unpredictable conditions elicited a positive attentional bias, whereas participants with low BAI scores only displayed a positive attentional bias in the uncontrollable-predictable condition (C-P+). To interpret these findings two things must be added: First, the reported attentional biases were calculated from no-danger trials to compare attentional biases towards threat as an indicator of anxiety without confounding danger cue induced fear. Additionally, comparing attentional biases in danger trials did not reveal any significant interaction effects (p>0.05). Second, negative attentional biases are generally seen as a coping mechanism of turning the attention away from threat in situations with aversive but not highly threatening stimuli (Cooper & Langton, 2006). In light of this, we speculate that in predictable conditions, both low anxious and high anxious individuals regulated their emotions in no-danger trials by turning their attention away from angry faces knowing that these trials signify periods of safety (see e.g., Grillon, 2002). In unpredictable conditions, on the other hand, participants had no periods of safety which led to sustained anxiety and positive attentional biases towards angry faces. Using this interpretation, the question remains why participants with low BAI scores still showed a negative attentional bias in the uncontrollable-unpredictable condition. However, it might be the case that these low anxious individuals comprehended (either consciously or subconsciously) that they could neither control nor predict shocks in this condition and thus turned to another emotion regulation strategy instead, whereas high anxious individuals failed to adapt such a strategy.

Our study has some limitations. The major limitation is that shock controllability was confounded with shock intensity in our experimental paradigm. Even though, all participants received the exact same number and same intensity of shocks (due to the within-subject design), the intensity of shocks was always lower in controllable compared to uncontrollable conditions in order to elicit the impression of control during controllable conditions. Previous studies admittedly demonstrated that subjective controllability had similar ameliorating effects on anxiety of pain and on pain perception (Salomons et al., 2004) as did

actual objective controllability. However, by using objective controllability in our study, we cannot rule out that the confounded shock intensity may have exaggerated the effects of uncontrollability in this experiment. For example, by examining the relative importance of the two factors on SC (C-P- > C-P+ > C+P- > C+P+), we found that controllability appears to have a greater impact on physiological arousal than predictability. However, this finding may be specific for our experimental design given that the implementation of controllability enabled the participants to ameliorate the aversive shocks, while predictability did not. Future studies should thus employ experimental designs allowing the combination of subjective controllability with predictability to avoid confounds with stressor intensity. Another limitation concerns our implementation of predictability. We modelled our experimental design based on Grillon's concept of predictability (Grillon et al., 2004, Mol et al., 2007). However, his concept of predictability slightly differs from the definition of predictability used in animal research. For instance, in our study, participants were explicitly informed about the imminent conditions, whereas rodents had to learn themselves which conditions are predictable or unpredictable. Additionally, in our predictable conditions, participants had to discriminate between cues signaling danger and cues signaling safety. Animals, in contrast, are usually only confronted with one signaling stimulus. These differences between human and animal research have to be considered when our results are interpreted. Furthermore, because we investigated heathy participants, we could not use clinical cut-offs to stratify our participants into groups of low vs. clinically relevant BDI/BAI scores. Using median splits to stratify our sample, however, might have led to arbitrary cut-offs so that our interaction results are likely not easy to replicate. Because of this, future studies should examine clinical samples applying clinically relevant cut-offs. Finally, we assumed in our interpretations that participants with higher depression scores also felt more helpless in the experiment and in their life circumstances. However, we did neither specifically assess their experimentally induced nor their general feelings of helplessness. Future studies should systematically assess feelings of helplessness during the experiment.

In conclusion, we provide evidence that uncontrollability and unpredictability of stressors have separate but also interacting effects on several anxiety measures in participants stratified according to subclinical scores of depression and anxiety. Previous research has already established that uncontrollable stress can elicit feelings of helplessness and depression, while unpredictable stress facilitates the emergence of sustained anxiety. However, our results reveal a more complex picture when the two factors are combined. Of course, our findings are preliminary at this point but they may turn out to be relevant considering the frequent co-morbidity of major depression and anxiety disorders. Stressful life experiences are often uncontrollable and unpredictable at the same time. According to our results, individuals who,

for example, already suffer from depression may be more susceptible to develop anxiety disorders in the face of stressors that are both uncontrollable and unpredictable. Neurobiological studies in animals suggest that such interactions might be processed in neural circuits involving connections between the amygdala and the dorsal raphe nucleus. Future studies could test this hypothesis using neuroimaging and a similar experimental paradigm as ours in clinical samples consisting of patients with depression, patients with anxiety disorders and patients with a depressive/anxiety co-morbidity. Additionally, epidemiological studies could examine the effects of stressful life experiences that are both uncontrollable and unpredictable in the development of co-morbid depression and anxiety disorders.

3 Study B: The neural underpinnings of prosexual effects induced by gammahydroxybutyrate in healthy humans

This chapter is an adapted version of the paper: Bosch OG*, Havranek MM*, Baumberger A, Preller KH, VonRotz R, Herdener M, Kraehenmann R, Stämpfli P, Scheidegger M, Seifritz E, Quednow BB. Neural underpinnings of prosexual effects induced by gamma-hydroxybutyrate in healthy humans. In submission. Contributions of Michael Havranek: took part in everything from designing the experiment to writing the publication. KEK-ZH-Nr. 2010-0357. * contributed equally to this publication.

3.1 Introduction

The idea of a love potion transforming a non-loving person into a loving one has exalted the imagination of innumerable alchemists, artists, and adventurers of all times. This notion has stimulated some of the most intriguing artworks such as the opera *Tristan and Isolde* by Richard Wagner, in which the knight Tristan falls in love with the princess Isolde after having been poisoned by her (Wagner, 1860). The quintessence of a love potion might be seen in its ability to alter the selection criteria of an individual by either creating previously inexistent affections for another person or by dissolving moral inhibitions, which are in conflict with already existing affections.

A contemporary view identifies the intention to pharmacologically induce erotic consent as a sexual assault, labelling an accordingly used substance as date-rape drug. In fact, the most frequently used rape drugs – alcohol and cannabis – have been proposed to mainly act via disinhibition of volitional control (Madea & Musshoff, 2009). Another drug discussed as an agent to facilitate sexual assaults is gamma-hydroxybutyrate (GHB), although it is actually used only in 0.2-4.4% of drug-related sexual assaults (Nemeth et al., 2010). However, abusers of the drug report prosexual effects including increased sexual desire, and decreased sexual inhibition (Lee & Levounis, 2008). Consequently occurring poor decision-making in erotic situations has been described as “lowering of sexual standards” for partner selection (Palamar et al., 2014).

GHB is an endogenous fatty acid and a metabolite of gamma-aminobutyrate (GABA)(Bessman & Fishbein, 1963). It appears to bind to specific GHB- (Benavides et al., 1982) and $GABA_B$-receptors (Engberg & Nissbrandt, 1993). Furthermore, it has neuromodulatory properties on glutamate, dopamine, serotonin, norepinephrine, and acetylcholine transmission (Andresen et al., 2011). Clinically, GHB is approved for the

treatment of narcolepsy and in some countries also for the treatment of alcohol withdrawal (Bosch et al., 2012).

The pharmacologic modulation of sexual arousal attracts growing scientific interest, as sexual dysfunction is increasingly recognized as illness- but also treatment-related impairment in several psychiatric conditions including schizophrenia and depression (Graf et al., 2014). However, while several experimental studies on the attenuating effects of psychotropic drugs exist (Graf et al., 2014), putative prosexual drug effects in humans are understudied. Methylphenidate elicits prosexual effects in a laboratory setting (Schmid et al., 2015, Volkow et al., 2007), while its neuronal underpinnings remain unknown. In contrast, apomorphine activates pre- and postcentral cortices, inferior parietal lobule (IPL), temporo-parietal junction (TPJ)(Montorsi et al., 2003), and the prefrontal cortex (Hagemann et al., 2003) during visual erotic stimulation, but subjective sexual arousal was not assessed in these studies.

Neuronal processing of visual erotic stimuli without pharmacologic challenge was studied in depth, and identified a canonical network consisting of cognitive (anterior cingulate cortex [ACC], pre- and postcentral gyrus, IPL, thalamus, insula), emotional (pre- and postcentral gyrus, amygdala, insula), motivational (precentral gyrus, ACC, hypothalamus, ventral striatum, IPL), and autonomic (ACC, hypothalamus, thalamus, insula) components (Kuhn & Gallinat, 2011, Stoleru et al., 2012).

In order to characterize putative prosexual effects of GHB and associated neuronal underpinnings, we performed two experiments in healthy male volunteers. The first experiment measured subjective and behavioral effects, using Sexual Arousal and Desire Inventory (SADI)(Toledano & Pfaus, 2006) and the Sexual Arousal Task (SAT)(Schmid et al., 2015) after oral administration of 25 and 35 mg/kg GHB vs. placebo to a total sample of 32 participants. In the second experiment, neural correlates of GHB-induced (35 mg/kg GHB) alteration of the perception of erotic vs. neutral visual stimuli were studied using functional magnetic resonance imaging (fMRI) in 19 participants. We hypothesized that GHB increases subjective and behavioral measures of sexual arousal and that an increased activation of the above mentioned functional network will occur during visual erotic stimulation under placebo and GHB.

3.2 Methods

Design and Participants

For both experiments, a randomized, double-blind, placebo-controlled, balanced, crossed within-subject design was used. Participants were heterosexual, non-smoking, healthy males. Experiment I: Thirty-two

participants with a mean age of 24.5 years (±3.8 *SD*, range 19-36), a mean verbal intelligence quotient (IQ) of 108.9 (±14.7, 86-145), and a mean weight of 74.9 kg (±8.3, 59-96). Experiment II: Nineteen participants with a mean age of 23.5 years (±3.6*,* 20-36), a mean verbal IQ of 113.4 (±18.4, 88-145), and a mean weight of 72.2 kg (±7.4, 59-85). Participants were recruited from online advertisings and underwent a medical and psychiatric examination applying the Structured Clinical Interview for DSM-IV Axis-I Disorders. Exclusion criteria were any DSM-IV psychiatric disorder, neurological disorder, severe medical disease, left-handedness, and regular illegal drug use (lifetime use > 5 occasions, with exception of occasional cannabis use), latter assessed using the Interview for Psychotropic Drug Consumption (Quednow et al., 2004) . Participants performed the Mehrfachwahl-Wortschatz-Intelligenztest (Lehrl, 1999) to estimate the verbal IQ and had to abstain from drinking alcohol 24h before the experiments and from drinking caffeinated beverages on the study days. Abstinence from illegal drugs was ensured with urine screenings (Dimension RXL Max, Siemens, Erlangen, Germany). The study was approved by the Cantonal Ethics Committee of Zurich and by Swissmedic and registered at ClinicalTrials.gov (NCT02342366). All participants provided written informed consent and were financially compensated.

EXPERIMENT I

Procedure

Participants randomly received either a low (20 mg/kg, n=16) or high dose (35 mg/kg, n=16) of GHB and placebo. Two test sessions were applied separated by seven days intervals. On the experimental days, participants had to fast during the morning and GHB (Xyrem® in orange juice) or placebo (salted orange juice) was given orally at 9:00am (t0min). Experimental sessions lasted for 225min.

Measures

Sexual Arousal and Desire Inventory: For sexual arousal assessment, we used a German version of the SADI consisting of 53 items (Toledano & Pfaus, 2006), which was implemented at time points: t-10min, t+50min, t+100min, and t+150min. Ratings are applied on a 5-point Likert-scale, ranging from 0 (“does not describe it at all”) to 5 (“describes it perfectly”). Four scales can be derived: *Evaluative*, *Negative/Aversive*, *Physiological*, and *Motivational* aspects of sexual arousal and desire.

Sexual Arousal Task: The SAT has been shown to be susceptible to acute effects of methylphenidate on emotional aspects of sexual perception (Schmid et al., 2015). It was implemented at time point t+40min.

It consists of sixteen pictures from the International Affective Picture System (Lang et al., 2008): eight sexually neutral pictures (four landscapes, four women), four implicit, and four explicit depictions of single women and couples. Stimuli were presented on a PC-screen in a randomized order for the duration of one second. Participants were able to prolong presentation time via mouse clicks (effort condition). Number of clicks and prolongation time were recorded. After stimuli presentation, participants had to rate each picture on a 9-point Likert-scale regarding the dimensions *pleasant*, *arousing/exciting*, *attractive*, *likeable*, and *erotic* (detailed description in: Schmid et al., 2015).

EXPERIMENT II

Procedure

Two sessions separated by seven days were applied. Participants completed an fMRI paradigm on a Philips Achieva 3T whole-body MR-unit equipped with a 32-channel head coil (Philips Medical Systems, Best, The Netherlands). The experiment started with a T1-weighted anatomical brain scan, baseline resting-state (rsfMRI) and arterial spin labeling (ASL). Subsequently, participants were taken out of the scanner and were orally administered with a single dose of GHB (35 mg/kg) or placebo (t0min). As t_{max} can be expected after about 40min, the fMRI paradigm began at t+30min. After a post-challenge rsfMRI/ASL scan, participants underwent the first run of the visual stimulation task (t+48min). After that, another rsfMRI/ASL scan was performed followed by the second run of the visual stimulation (t+70min). Subjective drug effects using Visual Analogue Scales (VAS) were assessed before visual stimulation (t+46min) and after stimulation runs (t+55min and t+77min) assessing *general drug effect, sedation, relaxation, stimulation, euphoria, body sensation, emotionality, sexual arousal, dizziness*, and *nausea.* At the end of the experiment final rsfMRI/ASL scans were assessed again after which participants were taken out of the scanner and debriefed. Experimental sessions lasted for 200min. rsfMRI/ASL data are not related to the present research question and will therefore be published elsewhere.

fMRI task

Participants were presented either neutral or erotic pictures of women or couples during two fMRI runs separated by 22min resulting in four experimental conditions: *placebo/neutral*, *placebo/erotic*, *GHB/neutral*, and *GHB/erotic*. One run of the task consisted of a total of 50 pictures presented for 4 seconds each in a blocked design (10 blocks, 20sec block duration) separated by fixation cross blocks (10

blocks, 20sec block duration). The pictures' order (neutral vs. erotic) was randomized between sessions and participants.

Data Acquisition

Functional time series were acquired with a sensitivity-encoded single-shot echo-planar imaging sequence (SENSE-sshEPI (Schmidt et al., 2005)). The fMRI protocol used the following acquisition parameters: TE=35ms, TR=2500ms (=82°), FOV=24cm, acquisition matrix=80x80 interpolated to 128x128, voxel size=3x3x3mm, 40 contiguous axial slices (placed along the anterior-posterior commissure plane), and SENSE factor R=2.0. For structural reference, a 3-dimensional T1-weighted anatomical scan with the following FFE sequence was obtained: TR/TE=9.3/4.6ms, flip angle=8°, 160 sagittal slices, FOV 240×240 mm, voxel size=1×1×1mm.

Data analysis

SADI data were analyzed using SPSS 22.0 for Windows, applying repeated measures ANOVA with drug (2-fold: GHB vs. placebo) and time (4-fold) as within-subject factors and dose (2-fold: high and low dose) as between-subject factor. SAT data were analyzed by a repeated measures ANOVA with the factors: *drug* (2-fold: GHB vs. placebo), *picture category* (3-fold: neutral, implicit sexual, explicit sexual), and drug dose (2-fold: high and low dose). Greenhouse-Geisser correction and adjusted p-values were used in models with more than one degree of freedom in the numerator. Paired t-tests were applied for post hoc treatment comparisons (placebo vs. GHB). All confirmatory statistical comparisons were carried out at a significance level of $p<.05$ (two-tailed).

fMRI data were analyzed using SPM8 (www.fil.ion.ucl.ac.uk/spm/). Motion artifacts were corrected by realignment to the mean image, mean-adjusted by proportional scaling, normalized according to the unified segmentation normalization approach (2x2x2mm) and spatially smoothed using a 6-mm full-with at half maximum Gaussian kernel. Statistical analysis was performed by modeling the four conditions (all minus fixation cross): *placebo/neutral*, *placebo/erotic*, *GHB/neutral*, and *GHB/erotic*, convolved with a hemodynamic response function as explanatory variables within the context of the general linear model on a voxel-by-voxel basis. Realignment parameters were included as additional regressors in the statistical model. A fixed-effect model at a single-subject level was performed to create images of parameter estimates, which were then entered into a second-level random effects analysis. For the fMRI data group

analysis, the contrast images from the analysis of the individual participants were analyzed using paired t-tests. Clusters of activation were identified with a global height threshold of $p<0.001$ (uncorrected) and a minimal threshold of $k>20$ voxels. Data from the VAS were analyzed using paired t-tests and results were considered significant if $p<.05$ (two-tailed).

3.3 Results

EXPERIMENT I

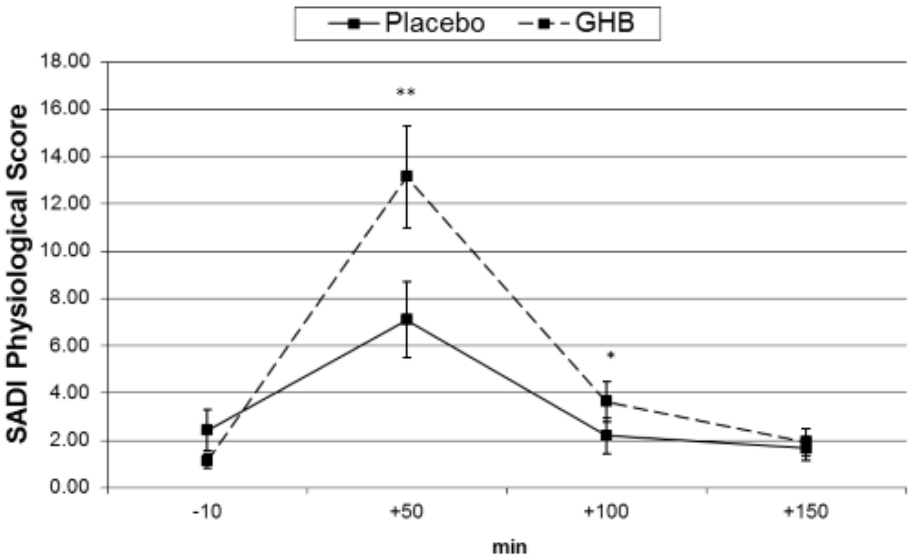
Sexual Arousal and Desire Inventory

Repeated measures ANOVAs (drug[2]*time[4]*dose[2]) revealed that GHB significantly increased ratings in the scales Physiological (drug: F[1,28]=3.55, p=.07; time: F[3,84]=37.2, p<.001; drug*time: F[3,84]=9.56, p<.001), Evaluative (time: F[3,84]=31.7, p<.001; drug*time: F[3,84]=7.87, p<.001), and Motivational (time: F[3,84]=22.4, p<.001; drug*time: F[3,84]=5.28, p<.01), while the scale Negative/Aversive did not differ (time: F(3,84)=1.60, p=.21; drug*time: F(3,84)=1.47, p=.24) compared to placebo. Post hoc t-tests confirmed significant drug effects at t+50min for the subscales Evaluative, and Motivational and at t+100min for the scale Physiological. As we did not find significant main effects for the factor dose (p>.25) on SADI measures, doses are shown pooled in Figure 4.

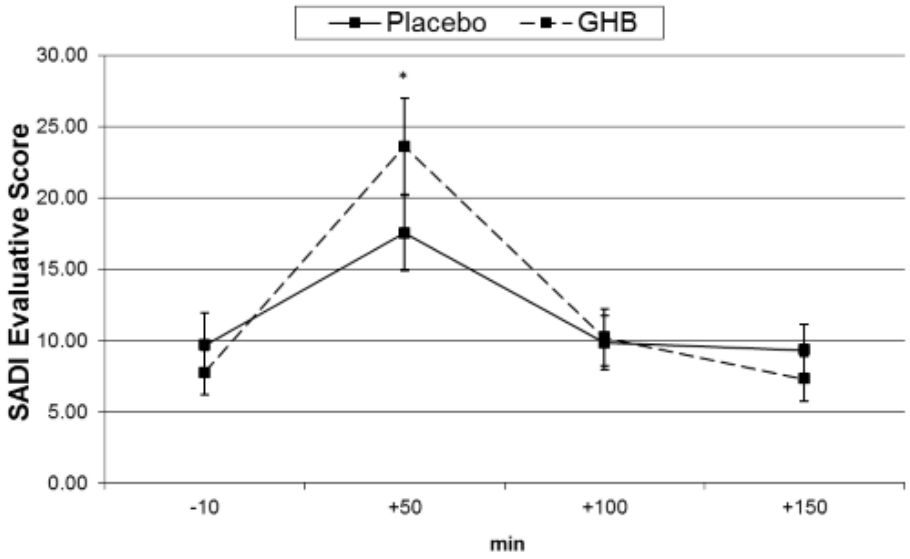
Sexual Arousal Task

Calculating repeated measures ANOVAs (drug[2]*picture category[3]*dose[2]) revealed only significant main effects for picture category in both the effort task (number of clicks: F(2,30)=8.61, p<.001; presentation prolongation: F(2,30)=8.47, p<.001) and the emotional ratings (e.g., for erotic: F(2,30)=154.50, p<.001). No drug, dose, or drug*picture category effects were found (Table 4).

A)


Placebo
GHB
SADI Physiological Score
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
**
*
-10
+50
+100
+150
min

B)


Placebo
GHB
SADI Evaluative Score
30.00
25.00
20.00
15.00
10.00
5.00
0.00
*
-10
+50
+100
+150
min

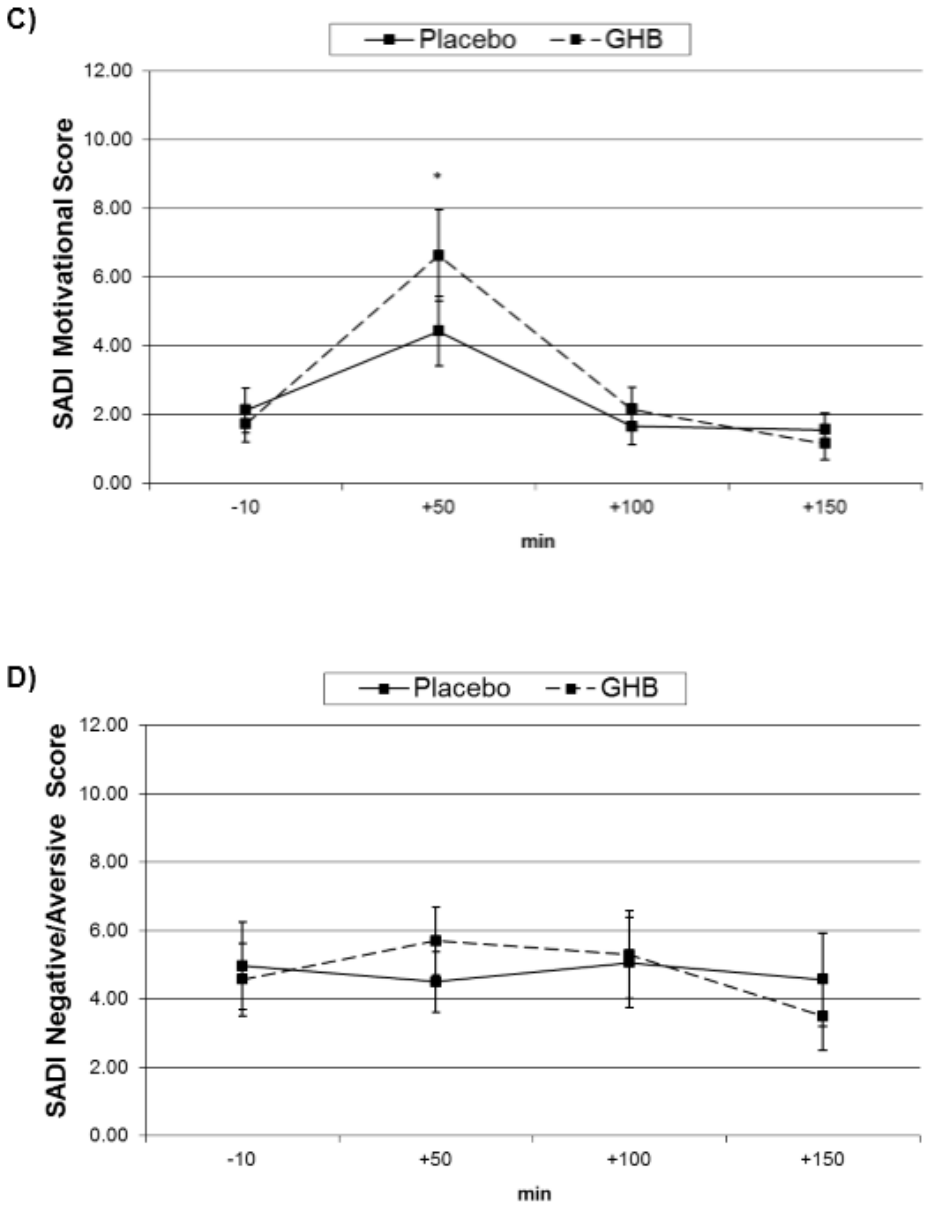


Figure 4. Ratings (means and *SEM*) of the scales Physiological (Fig. 4A), Evaluative (Fig. 4B), Motivational (Fig. 4C), and Negative/Aversive (Fig. 4D) of the Sexual Arousal and Desire Inventory (SADI) after GHB compared to placebo. Paired t-tests: $^{(*)}p<.10$, $^{*}p<.05$, $^{**}p<.001$.

Table 4. Drug effects in the sexual arousal task

	Placebo			**GHB Low Dose**			**GHB High Dose**		
	Neutral	Implicit	Explicit	Neutral	Implicit	Explicit	Neutral	Implicit	Explicit
Effort task									
Number of clicks	1.42 (1.97)	2.67 (3.02)**	2.32 (3.07)	2.16 (2.32)	4.13 (3.04)***	3.64 (3.26)	1.10 (1.74)	1.36 (2.04)	1.77 (2.27)
Duration (sec)	0.70 (0.87)	1.36 (1.42)**	1.06 (1.30)	1.04 (1.10)	1.88 (1.40)**	1.61 (1.33)	0.59 (0.83)	0.80 (1.02)	0.95 (1.21)$^{+}$
Arousal rating task									
Pleasant	5.58 (0.98)	6.75 (0.96)***	5.90 (1.20)	6.05 (0.80)	7.42 (0.89)***	6.41 (0.74)	5.24 (0.99)	6.34 (1.34)***	5.33 (1.40)
Arousing	2.91 (1.23)	5.26 (1.48)***	5.03 (1.74)***	3.40 (1.22)	6.05 (1.80)***	5.59 (1.60)***	2.89 (1.14)	4.64 (1.39)***	4.67 (1.73)**
Attractive	4.26 (1.06)	6.59 (1.06)***	5.48 (1.37)***	4.36 (0.92)	7.20 (0.88)***	6.00 (1.28)***	3.73 (1.09)	6.13 (1.50)***	5.09 (1.54)**
Likeable	4.69 (1.07)	6.02 (1.23)***	4.78 (1.28)	4.82 (1.01)	6.75 (0.94)***/$^{++}$	5.20 (1.44)	4.13 (1.13)	5.83 (1.31)***	4.58 (1.30)
Erotic	3.00 (1.05)	5.99 (1.28)***	5.71 (1.60)**	3.32 (1.02)	6.52 (1.15)***	6.27 (1.44)***	3.05 (0.96)	5.61 (1.50)	5.25 (1.79)***

Values are mean (*SD*) in 32 participants (placebo) and 16 participants (GHB Low vs. High Dose)

*p<0.05, **p<0.01, ***p<0.001 compared with respective neutral condition

$^{+}$<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 compared with respective placebo condition

EXPERIMENT II

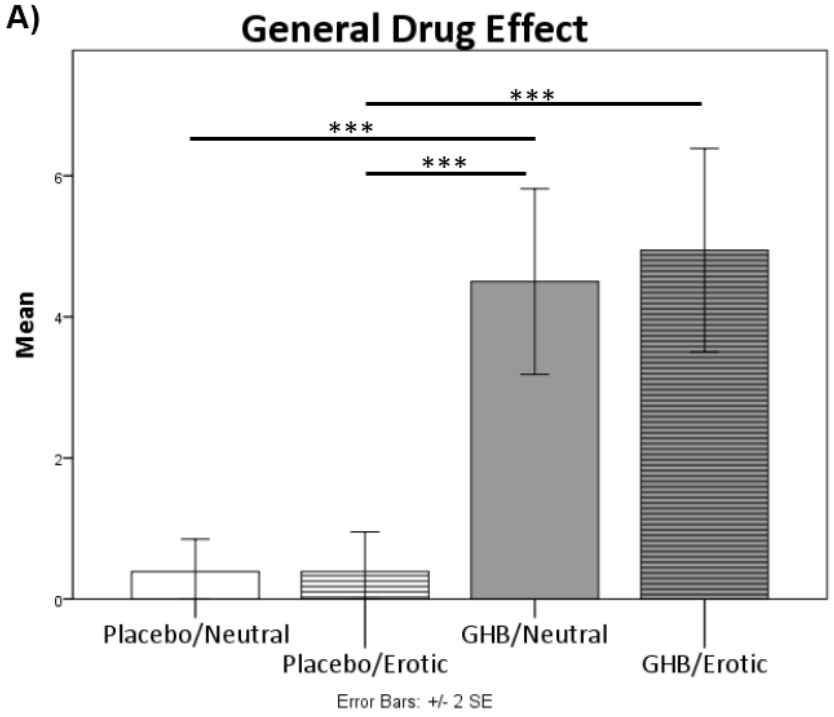
Subjective Measures

Comparing VAS results between GHB and placebo before task stimulation (t+46min) showed significant GHB-mediated increases in the VAS scales *general drug effects*, *sexual arousal, sedation, stimulation*, *euphoria*, *body sensation, emotionality, dizziness*, and *nausea* (all $p<0.001$, except for *body sensation* and *dizziness*: $p<0.01$ and *nausea*: $p<0.05$)(Table 5). After visual stimulation (t+55min and t+77min were pooled), we found that *general drug effect* and *sedation* clearly discriminated GHB and placebo conditions (both $p<0.001$)(Figure 5A/B), while there was a linear increase of *arousal* and *euphoria* over the four conditions (*placebo/neutral<placebo/erotic<GHB/neutral<GHB/erotic*)(Figure 5C/D). GHB increased *sexual arousal* for both erotic ($t(18)=3.22$, $p<0.01$) and neutral pictures ($t(18)=4.03$, $p<0.001$)(Figure 5E).

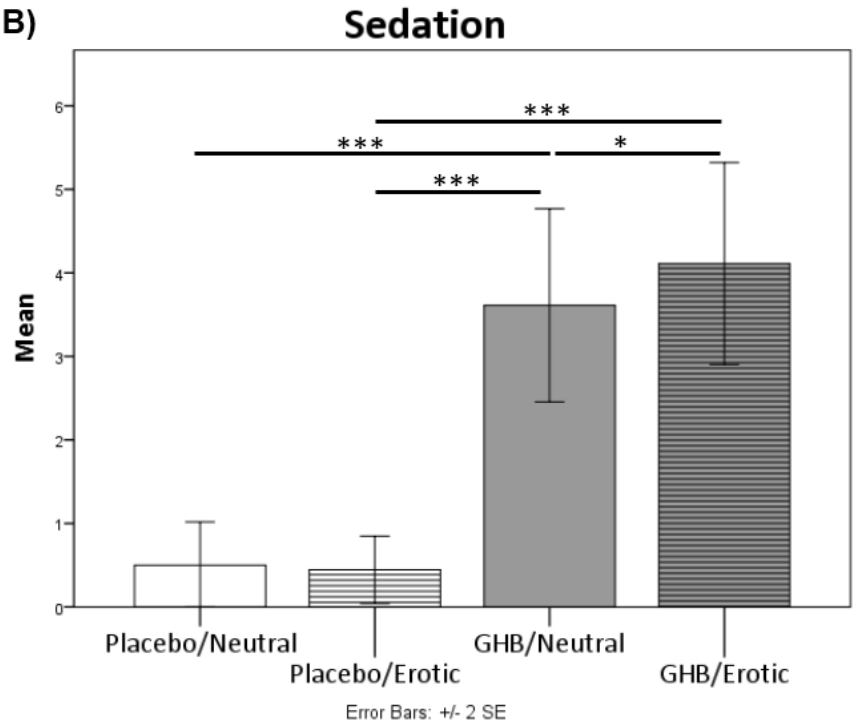
Table 5. VAS results before visual stimulation (means and *SD*)

	Placebo	**GHB**	***t-test***	***df***	***p***
General drug effect	0.32 (0.75)	6.00 (2.63)	8.64	18	**0.000**
Sexual arousal	0.63 (1.50)	2.11 (2.21)	3.83	18	**0.001**
Sedation	0.42 (1.02)	4.79 (2.25)	8.69	18	**0.000**
Stimulation	1.42 (1.87)	4.47 (2.67)	5.05	18	**0.000**
Euphoria	1.26 (1.37)	4.11 (2.54)	5.65	18	**0.000**
Body sensation	4.21 (2.44)	5.89 (2.23)	3.08	18	**0.006**
Emotionality	3.74 (2.77)	5.89 (2.81)	5.51	18	**0.000**
Relaxation	5.16 (2.73)	6.26 (1.82)	1.59	18	0.130
Dizziness	0.47 (1.10)	2.53 (2.70)	3.71	18	**0.002**
Nausea	0.21 (0.71)	1.26 (2.28)	2.45	18	**0.025**

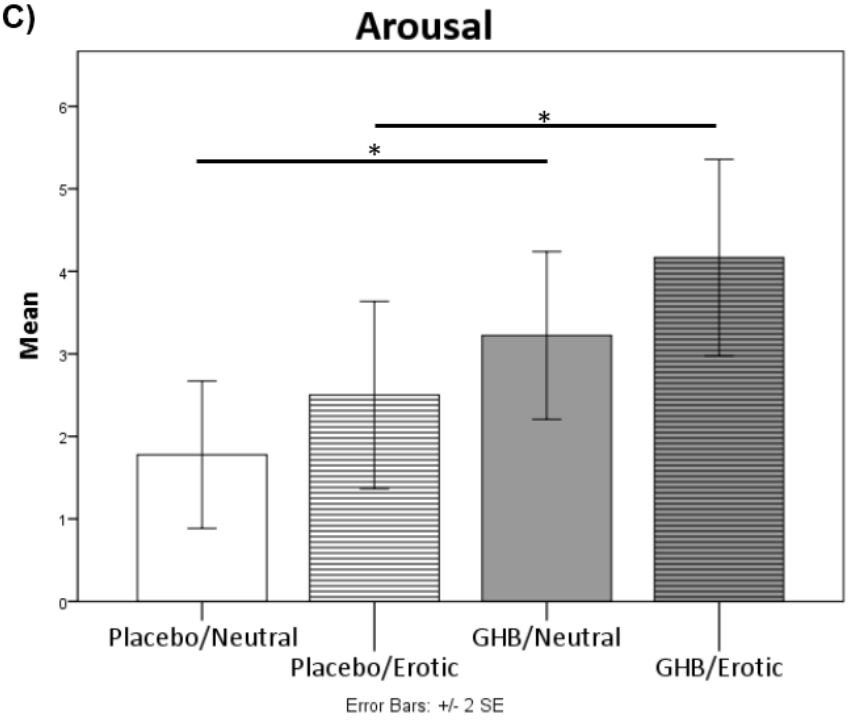
A)


General Drug Effect
***
***
***
Mean
0
2
4
6
Placebo/Neutral
Placebo/Erotic
GHB/Neutral
GHB/Erotic
Error Bars: +/- 2 SE

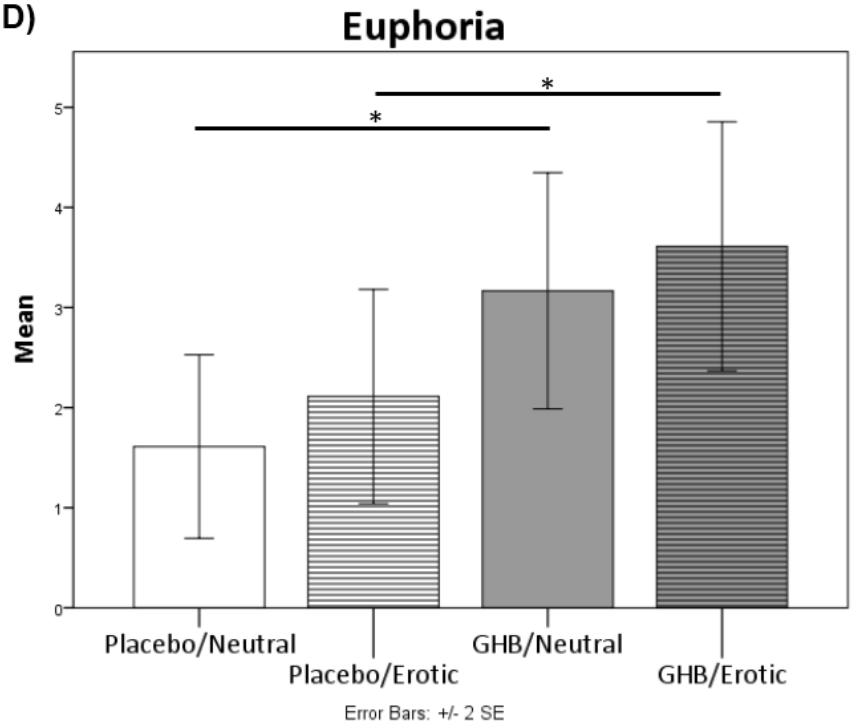
B)


Sedation
***
***
*
***
Mean
0
1
2
3
4
5
6
Placebo/Neutral
Placebo/Erotic
GHB/Neutral
GHB/Erotic
Error Bars: +/- 2 SE

C)


Arousal
Mean
0
1
2
3
4
5
6
*
*
Placebo/Neutral
Placebo/Erotic
GHB/Neutral
GHB/Erotic
Error Bars: +/- 2 SE

D)


Euphoria
Mean
0
1
2
3
4
5
*
*
Placebo/Neutral
Placebo/Erotic
GHB/Neutral
GHB/Erotic
Error Bars: +/- 2 SE

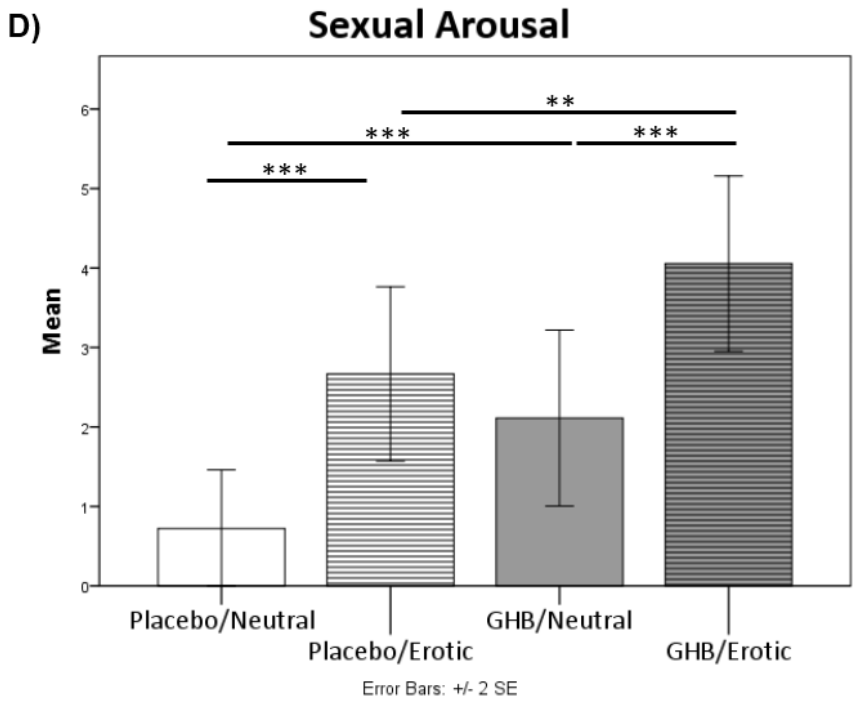


Figure 5. Visual analogue scale (VAS) ratings after visual stimulation task (t+55min and t+77min were pooled) in the conditions placebo/neutral, placebo/erotic, GHB/neutral, and GHB/erotic: general drug effect (Fig. 5A), sedation (Fig. 5B), arousal (Fig. 5C), euphoria (Fig. 5D), and sexual arousal (Fig. 5E). Paired t-tests: *p<.05, **p<.01, ***p<.001.

Neuroimaging

Pairwise comparisons of the whole brain statistical maps between erotic and neutral pictures in the placebo condition (erotic>neutral under placebo: *placebo/erotic*) revealed significant BOLD response increases in the left ACC (Figure 3_cluster a), left precentral (Figure 3_b) and right postcentral (Figure 3_c) gyri, left IPL (Figure 3_d), right thalamus (Figure 3_e), right inferior temporal gyrus (Figure 3_g), left inferior (Figure 3_h) and bilateral middle occipital gyrus (Figure 3_i), and left pallidum (Figure 3_j)(all p<0.001, k=20, Table 3 and Figure 3, red).

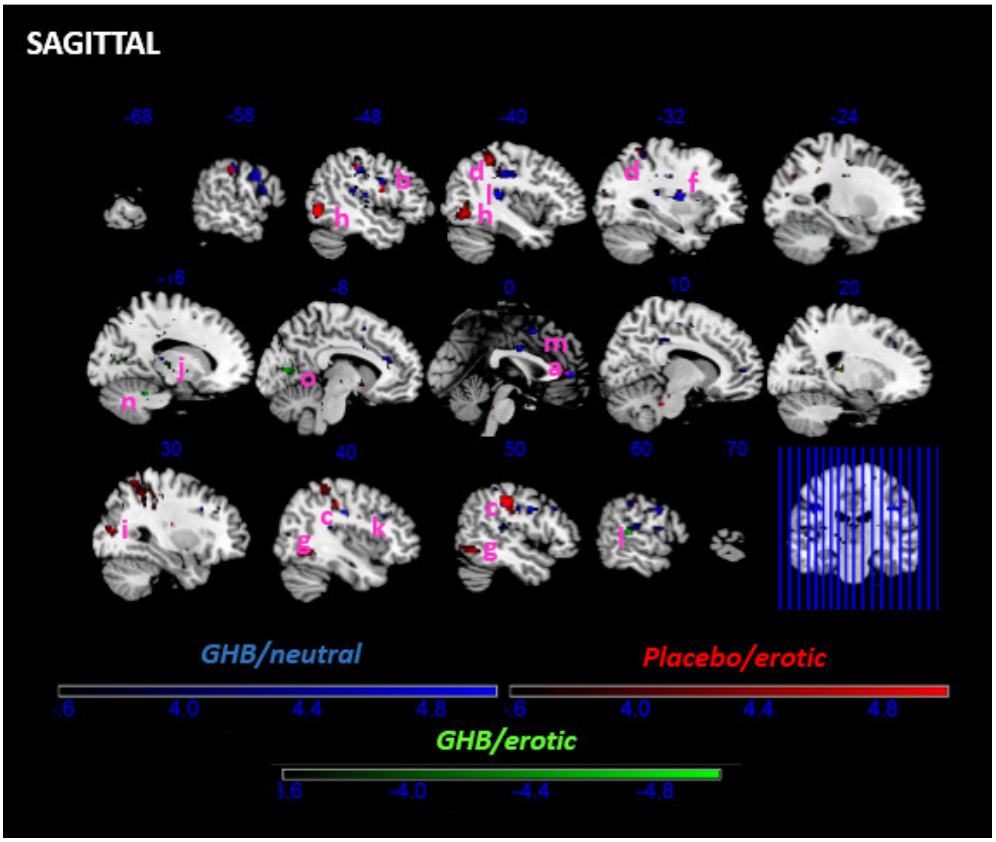
In response to neutral pictures, GHB (GHB>placebo in neutral condition: *GHB/neutral*) elicited significant BOLD response increases in the bilateral middle cingulate cortex and ACC (Figure 6A), left precentral (Figure 6B) and bilateral postcentral (Figures 6C) gyri, right IPL (Figures 6D), left thalamus (Figures 6E), left insula (Figures 6F), right middle frontal gyrus (Figure 6K), bilateral superior temporal gyrus (Figure 6L), and left supplementary motor area (SMA)(Figure 6M)(all p<0.001, k=20, Table 6 and Figure 6, blue).

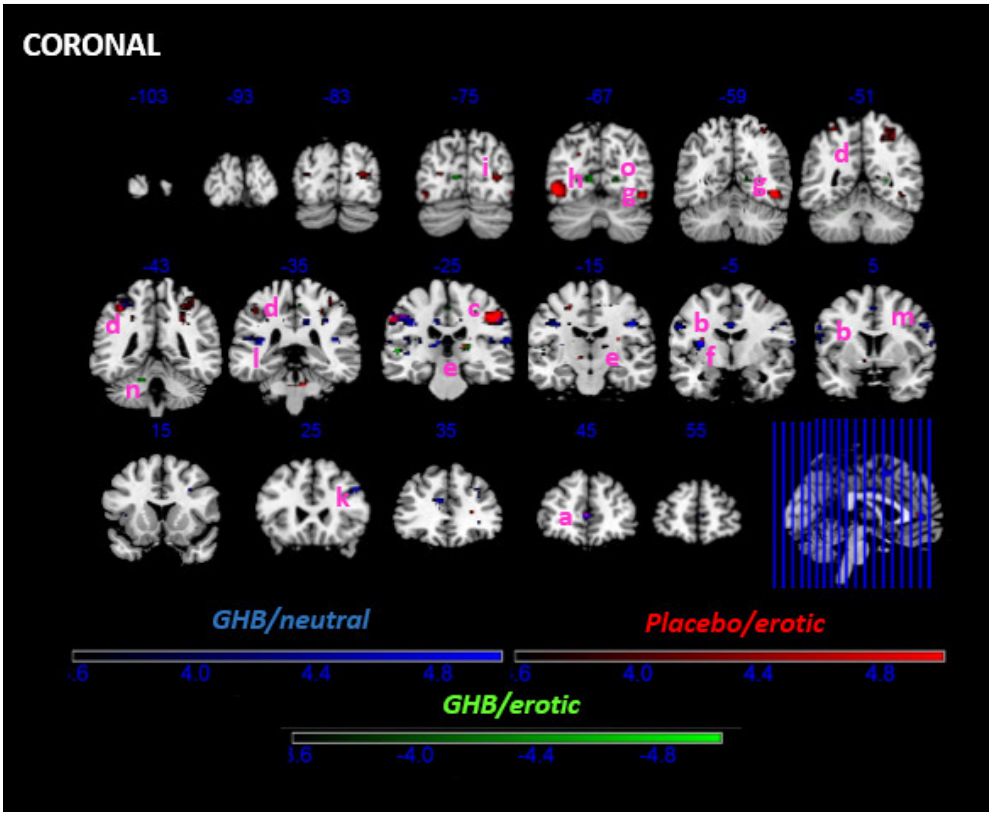
The comparison of GHB>placebo in the erotic condition (*GHB/erotic*) revealed diminished activation of the bilateral thalamus (Figures 6E), bilateral superior temporal gyrus (Figure 6L), left anterior lobe of cerebellum (Figure 6N), and bilateral calcarine gyrus (Figure 6O)(all $p<0.001$, $k=20$, Table 6 and Figure 6, green).

Table 6. Peak MNI coordinate regions with significant BOLD signal changes

Peak MNI coordinate regions	Laterality	x, y, z (MNI)	*t* value	Cluster size	Figure
Erotic > neutral in the placebo condition, only BOLD increases were found ($p<0.001$, $k=20$)					
Anterior Cingulate Cortex	L	0 44 6	5.1645	20	3a, 4a
Precentral Gyrus	L	-50 0 20	5.3695	38	3b, 4b
Postcentral Gyrus	R	46 -28 42	7.1621	838	3c, 4c
Inferior Parietal Lobule	L	-40 -42 50	5.6696	386	3d, 4d
Thalamus	R	14 -14 8	4.4197	21	3e, 4e
Inferior Temporal Gyrus	R	46 -66 -8	6.036	267	3g
Inferior Occipital Gyrus	L	-48 -68 0	5.5999	349	3h
Middle Occipital Gyrus	Bilateral	28 -80 10	4.7736	179	3i
Pallidum	L	-14 -2 -4	4.2817	21	3j
GHB > placebo in the neutral condition, only BOLD increases were found ($p<0.001$, $k=20$)					
Anterior and Middle Cingulate Cortex	Bilateral	0 -6 32	5.0149	90	3a, 4a
Precentral Gyrus	L	-58 0 32	5.5781	313	3b, 4b
Postcentral Gyrus	Bilateral	-38 -26 36	4.8651	408	3c, 4c
Inferior Parietal Lobule	Bilateral	56 -32 42	4.743	191	3d, 4d
Thalamus	L	-14 -28 12	5.0832	36	3e, 4e
Insula	L	-32 -6 14	5.6652	126	3f, 4f
Middle Frontal Gyrus	R	46 24 34	4.8689	152	3k
Superior Temporal Gyrus	Bilateral	-40 -32 14	6.08	404	3l
Supplementary Motor Area	L	-4 8 52	5.7547	107	3m
GHB > placebo in the erotic condition, only BOLD reductions were found ($p<0.001$, $k=20$)					
Thalamus	Bilateral	-18 -22 4	-4.2919	21	3e, 4e
Superior Temporal Gyrus	Bilateral	60 -32 12	-5.0114	59	3l
Anterior Lobe of Cerebellum	L	-16 -42 -28	-5.0873	56	3n
Calcarine Gyrus	Bilateral	-10 -72 10	-4.8905	123	3o

R = right hemisphere, L = left hemisphere, MNI = Peak MNI coordinates, cluster sizes are in voxels


SAGITTAL
-68
-58
-48
-40
-32
-24
-18
-8
0
10
20
30
40
50
60
70
GHB/neutral
Placebo/erotic
4.0
4.4
4.8
GHB/erotic
-4.0
-4.4
-4.8


CORONAL
-103
-93
-83
-75
-67
-59
-51
-43
-35
-25
-15
-5
5
15
25
35
45
55
GHB/neutral
Placebo/erotic
4.0
4.4
4.8
GHB/erotic
-4.0
-4.4
-4.8

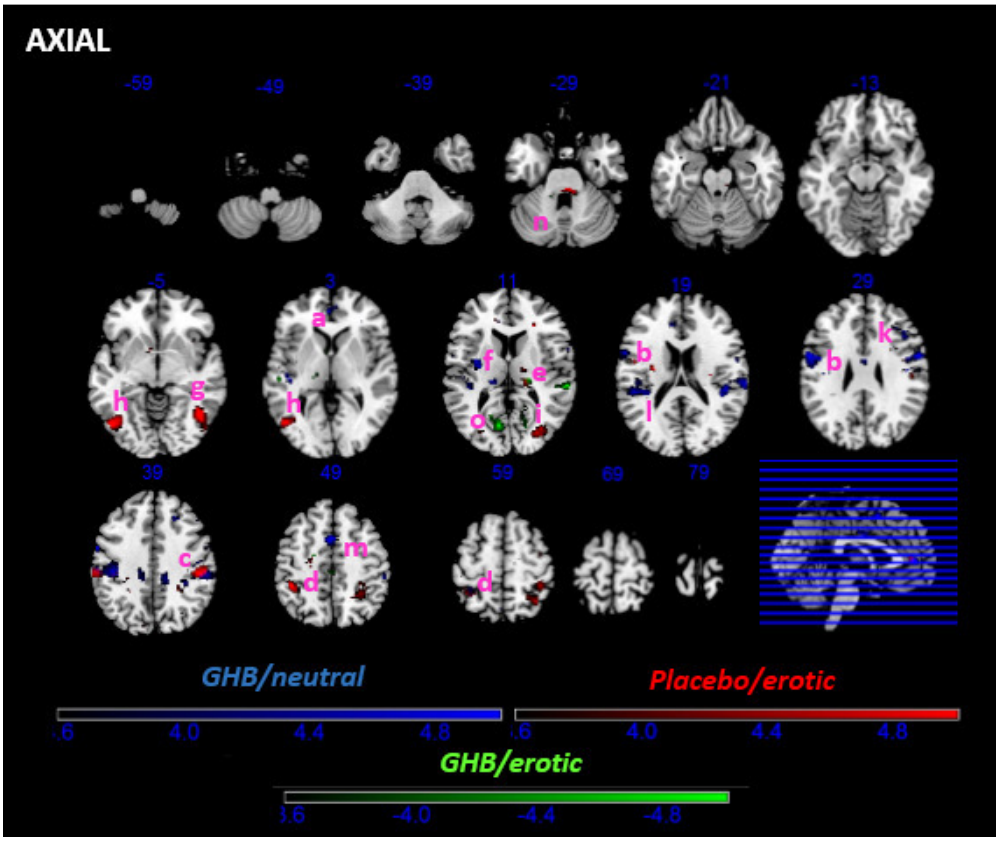
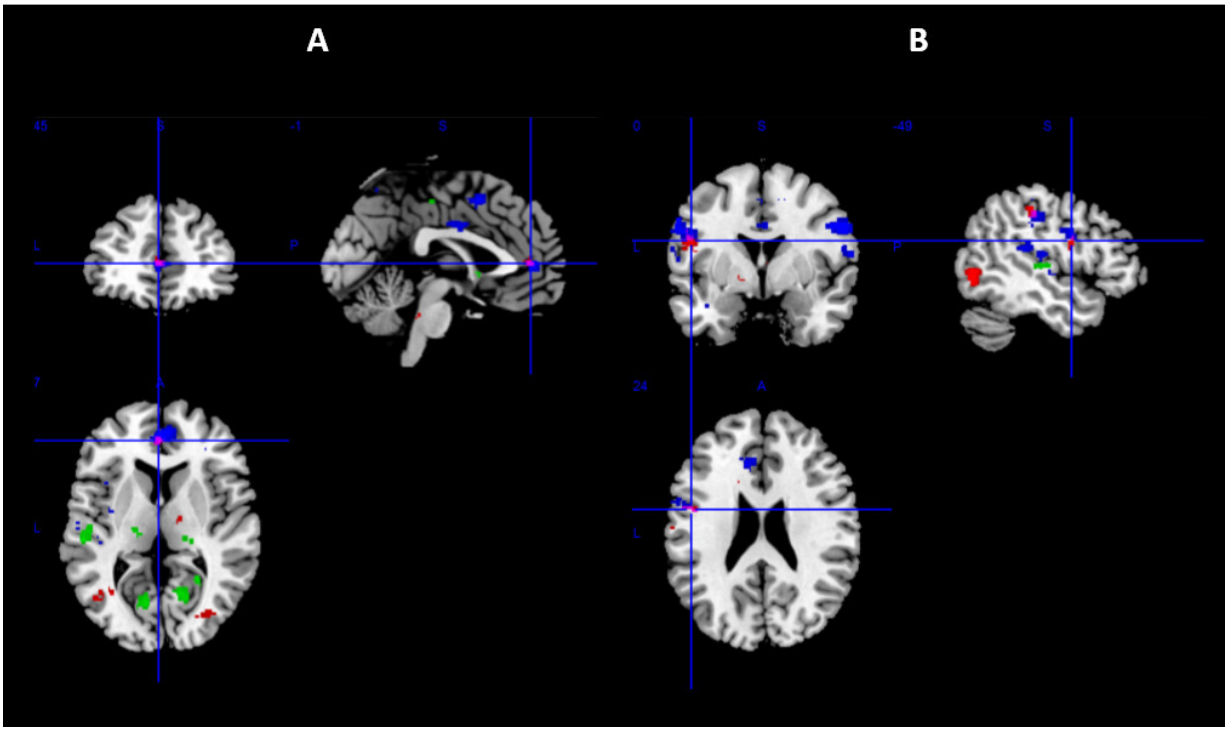
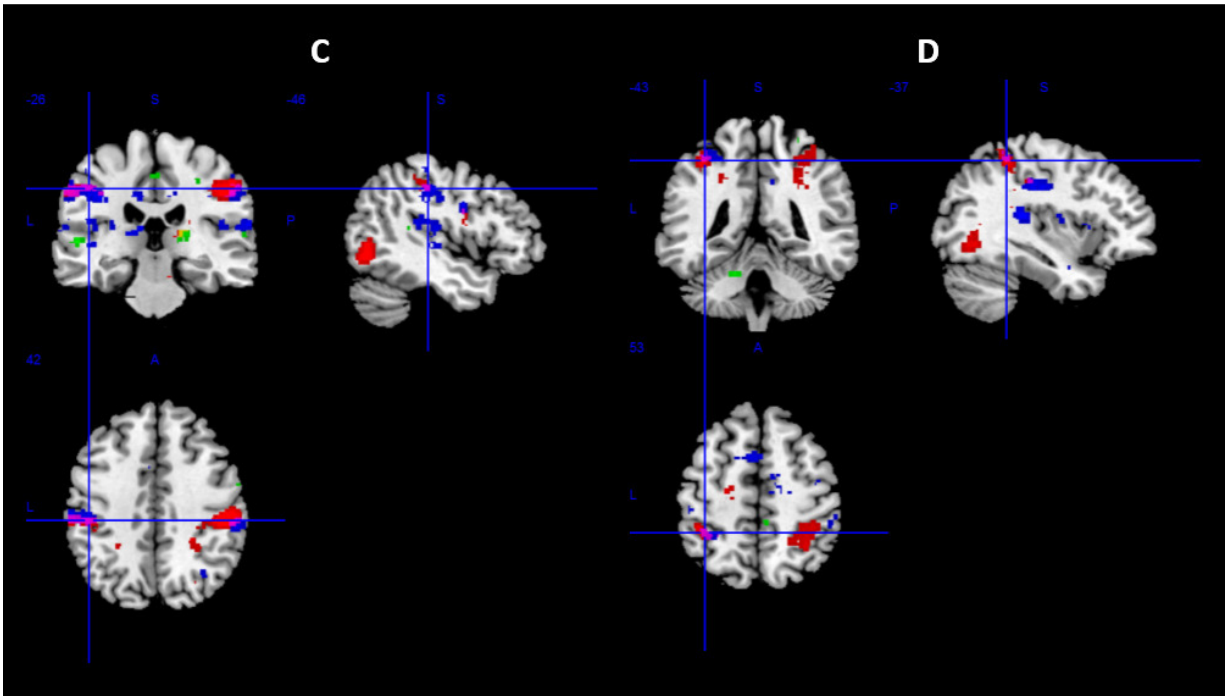


Figure 6. Overlay BOLD response pattern map. Color codes: red - placebo/erotic; blue - GHB/neutral; green - GHB/erotic; pink – overlay of placebo/erotic and GHB/neutral; yellow - overlay of placebo/erotic and GHB/erotic. Significant clusters: anterior cingulate cortex (Fig. 6A), precentral gyrus (Fig. 6B), postcentral gyrus (Fig. 6C), inferior parietal lobule (Fig. 6D), thalamus (Fig. 6E), insula (Fig. 6F), inferior temporal gyrus (Fig. 6G), inferior occipital gyrus (Fig. 6H), middle occipital gyrus (Fig. 6I), pallidum (Fig. 6J), middle frontal gyrus (Fig. 6K), superior temporal gyrus (Fig. 6L), supplementary motor area (Fig. 6M), anterior lobe of cerebellum (Fig. 6N), calcarine gyrus (Fig. 6O). Clusters of activation were identified with a global height threshold of $p<0.001$ (uncorrected) and a minimal threshold of $k>20$ voxels.

Intriguingly, there was a strong overlap of patterns of *placebo/erotic* and *GHB/neutral* (Figure 7), which were both paralleled by increased sexual arousal (Figure 5E). Overlaps are depicted in Figure 7: bilateral ACC (Fig. 7A), bilateral precentral gyri (Fig. 7B), bilateral postcentral gyri (Fig. 7C), and bilateral IPL (Fig. 7D), and an overlap of *placebo/erotic* and *GHB/erotic* in the left thalamus (Fig. 7E).


A
B
45
S
-1
S
L
P
7
A
L
0
S
-49
S
L
P
24
A
L


C
D
-26
S
-46
S
L
P
42
A
L
-43
S
-37
S
L
P
53
A
L

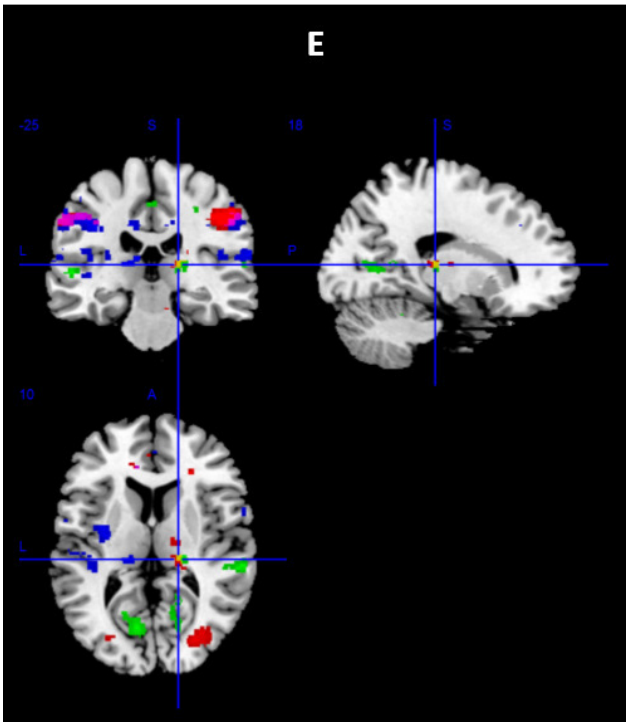


Figure 7. Overlay BOLD response foci: anterior cingulate cortex (Fig. 7A), precentral gyrus (Fig. 7B), postcentral gyrus (Fig. 7C), inferior parietal lobule (Fig. 7D), thalamus (Fig. 7E): red - placebo/erotic; blue - GHB/neutral; green - GHB/erotic; pink – overlay of placebo/erotic and GHB/neutral; yellow - overlay of placebo/erotic and GHB/erotic. Clusters of activation were identified with a global height threshold of $p<0.001$ (uncorrected) and a minimal threshold of $k>20$ voxels.

3.4 Discussion

The present study is the first investigating the neural effects of GHB using brain imaging techniques. Our results suggest that GHB in fact has prosexual effects in healthy males as the drug induced and increased sexual arousal with and without visual erotic stimulation, respectively. However, emotional ratings of sexually neutral, implicit, and explicit pictures presented in the SAT remained unaffected by the drug. In a sexual stimulation fMRI paradigm, erotic pictures under placebo (*placebo/erotic*) elicited sexual arousal and increased BOLD signals in a neuronal network comprising ACC, pre- and postcentral gyri, IPL, and the thalamus. Intriguingly, after GHB intake, this network was also activated by neutral pictures (*GHB/neutral*), paralleled by increased sexual arousal. In this condition, additional activations in the insula, middle frontal and cingulate gyri and SMA could be found. However, the combination of GHB and erotic pictures (*GHB/erotic*) resulted in an attenuating effect leading to less pronounced activation in the thalamus, superior temporal gyrus, anterior lobe of cerebellum, and the calcarine gyrus, while subjective sexual

arousal was highest in this condition. Whereas most overlaps occurred between *placebo/erotic* and *GHB/neutral*, converging modulation was found in the thalamus for all three above mentioned conditions, and in the superior temporal gyrus/TPJ for *GHB/neutral* and *GHB/erotic*, pointing to a localized GHB-specific effect.

Used as a club drug, GHB was reported to induce intense sexual feelings leading to "lower sexual standards" for partner selection (Palamar et al., 2014). Here, GHB increased sexual arousal, resulting in elevated ratings in the SADI-scales *Physiological*, *Evaluative,* and *Motivational* (Figure 1a-c). Visual stimulation with erotic, but also with neutral pictures of persons increased VAS for sexual arousal after GHB intake (Figure 2e). Volkow and colleagues (2007) demonstrated increased sexual desire after 0.5mg/kg methylphenidate, while Schmid *et al* (2015) found a methylphenidate-induced increase in ratings of sexual arousal in the here used SAT, both confirming reports of sexual enhancing effects of psychostimulants. In contrast to methylphenidate, GHB robustly increased subjective sexual arousal in our self-rating scales, while emotional ratings of sexual stimuli in the SAT remained unaffected. The prosexual effects of psychostimulants are attributed to their capacity to increase dopamine concentrations in the mesolimbic reward system consisting of dopaminergic neurons in the ventral tegmental area (VTA) that innervate the nucleus accumbens (NAcc), amygdala, and the medial prefrontal cortex (mPFC)(Frohmader et al., 2010). Contrary, GHB is a mixed GHB-/$GABA_B$ agonist but its behavioral effects are mediated mostly by GABAergic mechanisms when exogenously applied (Carter et al., 2009). The only GABAergic drug discussed as potentially prosexual is alcohol but animal and human data show conflicting results, indicating a sexually disinhibiting effect of low doses of alcohol (Frohmader et al., 2010). However, GHB also has downstream effects on the neurotransmission in the mesolimbic dopamine system (Snead & Gibson, 2005). In the substantia nigra and striatum of rats the occurrence of markers for GHB synthesis indicates GHBergic control of presynaptic dopaminergic activity (Hedou et al., 2000). However, it is a matter of debate if GHB enhances (Cruz et al., 2004) or inhibits (Brancucci et al., 2004) dopaminergic output in terminal regions, and if these effects are mediated by $GABA_B$- or GHB-receptors. Converging evidence points to a dose- and time-dependent bi-directional effect of GHB on mesolimbic dopamine release (Hechler et al., 1991). Lower doses seem to indirectly disinhibit dopaminergic VTA neurons, while increasing doses additionally directly inhibit dopaminergic neurons (Labouebe et al., 2007). Thus, the here found GHB-induced prosexual effects might be explained by its disinhibiting effects on VTA neurons, resulting in increased dopamine release in the NAcc and mPFC.

In our participants, erotic pictures in the placebo condition and neutral pictures in the GHB condition elicited sexual arousal and activated neuronal networks with considerable overlap in ACC, precentral and

postcentral gyri, IPL, and thalamus (Figure 4). The network identified here, highly corresponds to a canonical sexual-cue processing network anteriorly described in the meta-analyses of Kuhn and Gallinat (2011) and Stoleru *et al*. (2012). Specifically, our pattern covers primarily cognitive (ACC, pre- and postcentral gyrus, IPL, thalamus), but also emotional (pre- and postcentral gyrus), motivational (precentral gyrus, ACC, IPL), and autonomic (ACC, thalamus) components outlined by these authors. In fact, the central dopamine receptor agonist apomorphine, which is used as second-line treatment for erectile dysfunction, also increased activity in ACC (Hagemann et al., 2003), pre- and postcentral gyrus, IPL, and TPJ (Montorsi et al., 2003) in response to visual erotic stimulation, but unfortunately subjective sexual arousal has not been assessed in these studies.

The ACC mediates motivational aspects of behavior including sexual arousal (Sewards & Sewards, 2003). It is regarded as a key structure in the processing of the initiation, persistence, and seeking of sexual reward (Frohmader et al., 2010). In humans, the ACC is crucial for the processing of emotional valence of erotic stimuli (Walter et al., 2008). Moreover, reduced ACC responsiveness to erotic stimuli was found under medication with paroxetine, which was paralleled by increased subjective ratings of sexual dysfunction. In another fMRI study, resting-state functional connectivity reductions of the amygdala with the ACC, dopaminergic midbrain, and insula predicted the occurrence of paroxetine-induced sexual dysfunction, whereby ACC connectivity was correlated with impaired sexual satisfaction (Metzger et al., 2013). Here, enhancement of ACC activity might be a useful biomarker to assess GHB effects on hedonic sexual functioning, including potential therapeutic implications for depression treatment and resolution of antidepressant-related sexual dysfunction.

The commonly seen activation of pre- and postcentral cortices under visual erotic stimulation has been interpreted as a correlate of somatosensory imagery (Stoleru et al., 2012). When participants observe somatic experiences of others, their own somatosensory cortices are activated (Keysers et al., 2010), which particularly applies to sexual imagery. We found a highly overlapping cluster of pre- and postcentral activation in *placebo/erotic* and *GHB/neutral* (Figure 3/ 4b,c), both of which induced sexual arousal. Another parietal area, which is typically activated by erotic cues is the IPL, which is involved in maintaining attention to motivationally relevant targets (Stoleru et al., 2012). Underlining the concept of parietal processing of somatosensory imagery, the IPL forms part of the human mirror-neuron system, which is an interface between perceptual and motoric representations of actions (Chong et al., 2008). Moreover, activation of IPL and frontal operculum by erotic video-clips predicted penile rigidity, indicating that these areas not only process motor correlates of observed actions, but also corresponding autonomic aspects (Mouras et al., 2008). Moreover, the opercular part of the superior temporal gyrus showed overlapping

modulation between *GHB/neutral* and *GHB/erotic*, suggesting a GHB-specific effect. In a recent meta-analysis, this area (also termed as TPJ) was shown to process reorientation to salient stimuli and Theory-of-Mind (ToM) during social interactions (Krall et al., 2015), pointing to a potential enhancing effects of the drug on these functions. In fact, we recently demonstrated prosocial effects of GHB in humans, while ToM remained unchanged (Bosch et al., 2015). Moreover, the TPJ is strongly connected to the postcentral gyrus, IPL, and the thalamus. A GHB-specific modulation of this area could facilitate sexual processing of otherwise neutral stimuli, as it influences the here identified sexual-cue network. Intriguingly, this network shares features with a mirror-neuron network incorporating pre- and postcentral gyri, IPL, and the occipito-temporal junction (Molenberghs et al., 2012). It was postulated that the parietal lobe matches visual information to internal drive states such as goal-directed behavior (Lynch et al., 1977). The involvement of the human mirror-system in the processing of erotic stimuli seems to confirm this hypothesis, highlighting the importance of sexual behavior in the set of human conduct. In our study, GHB-induced sexual arousal independently from explicit sexual stimulation, led to an activation of the sexual-cue related mirror-system (*GHB/neutral*)(Figure 4b,c,d). It is likely, that the shift of the internal drive state induced a lowering of the threshold for the interpretation of visual stimuli as erotic, a process that might be mediated by parietal mirror-neuron areas.

The posterolateral part of the thalamus was the only structure where we found an overlap in all three conditions *placebo/erotic*, *GHB/neutral* and *GHB/erotic* (Figure 3/4e). Representations of sexual need in animals are represented in the thalamus (Sewards & Sewards, 2003). In human males, the ventroposterior nucleus was one of three thalamic nuclei which showed an increased regional cerebral blood flow (rCBF) during ejaculation (Holstege et al., 2003). Moreover, high resolution fMRI showed an activation of the mediodorsal thalamus in reaction to visual erotic stimuli in humans (Walter et al., 2008). Erotic stimuli-induced thalamic activation was commonly interpreted as reflecting general emotional arousal, but some studies which found correlations with penile rigidity point to more specific role in sexual stimuli processing (Stoleru et al., 2012).

Interestingly, the condition *GHB/erotic* led to the highest levels of self-described sexual arousal, while our neuroimaging results showed a less pronounced activation in the thalamus, superior temporal gyrus, left anterior part of the cerebellum, and calcarine gyrus compared to *placebo/erotic*. Notably, a correlation of decreased rCBF in the same cerebellar area with penile rigidity after erotic stimulation and apomorphine challenge was found anteriorly (Hagemann et al., 2003). In the first place, this effect might seem paradoxical, as a linear relationship between subjective effects and neuronal pattern activation would have been expected. However, a possible explanation could be that GHB facilitates sexual-cue processing,

and that the presentation of pictures with already explicit sexual content produce less computational load and therefore result in lower activation. Such neuronal recruitment differences can also be found in speech processing, as the production of native speech sounds requires a reduced neural recruitment compared to novel speech (Moser et al., 2009). In our participants, the semantic reinterpretation of non-erotic/neutral pictures towards an erotic perception under the influence of GHB might need higher neural recruitment, while the facilitation of the erotic perception of an already explicitly sexual stimulus might need a less intense neural recruitment. This interpretation is speculative but might offer a plausible explanation for our non-linear results.

Our study bears the limitation that we only assessed heterosexual males. This was owed to the pragmatism that we wanted to start our design with one gender and one sexual orientation first.

In summary, our primary finding is that GHB induces sexual arousal and lowers the threshold for erotic perceptions. This means that under GHB, otherwise non-erotic stimuli induce sexual arousal and activate a sexual-cue related brain pattern including ACC, pre- and postcentral gyri, IPL, and thalamus. A GHB-specific effect was observed on the TPJ, an area that is highly involved in the processing of social interactions. The study, thus, confirms recreational GHB users' reports of enhanced sexual arousal and less careful sexual partner selection at an objective, experimental level. The observed prosexual effects may be elicited by an activation of the mesolimbic reward system due to GHB- and/or $GABA_B$-receptor stimulation. Sexual dysfunction is an important dimension across several psychiatric diagnostic entities and should be considered as a specific therapy target. Therefore, the here demonstrated enhancement of hedonic sexual functioning makes GHB an interesting experimental therapeutic in psychiatric conditions which are accompanied by anhedonia such as schizophrenia and depression.

4 Study C: α_{2A}-adrenergic receptor polymorphisms are associated with delay discounting in cocaine users

This chapter is an adapted version of the paper: Havranek MM, Hulka LM, Tasiudi E, Eisenegger C, Vonmoos M, Preller KH, Mössner R, Baumgartner MR, Seifritz E, Grünblatt E, Quednow BB. α2A-adrenergic receptor polymorphisms and mRNA expression levels are associated with delay discounting in cocaine users. Addict Biol. In press. Contributions of Michael Havranek: everything from data analysis to writing the publication (excluding data acquisition).

4.1 Introduction

Maladaptive decision-making has been suggested as a core feature in stimulant addiction as cocaine users characteristically seek the instantaneous and brief reinforcement of the drug despite its negative future consequences (Hulka et al., 2014, Koob, 2009, MacKillop, 2013). At the basis of this behavior lies an impulsive preference for smaller immediate rewards over larger delayed rewards coined as delay discounting (DD). Several studies provided evidence that cocaine users display higher discounting rates for future rewards compared to stimulant-naïve controls (Bickel et al., 2011, Coffey et al., 2003, Heil et al., 2006, Hulka et al., 2014, Kirby & Petry, 2004). In addition, stronger DD has not only been associated with higher levels of drug consumption and poor treatment response in cocaine users but also with general negative outcomes in financial, professional, and health domains (Brody et al., 2014, Mischel et al., 2011, Moffitt et al., 2011, Washio et al., 2011). However, despite the importance of DD in cocaine addiction, its molecular underpinnings remain unclear so far.

Evidence from human and animal studies suggests the involvement of the norepinephrine (NE) system and more specifically the α_{2A}-adrenergic receptor (*ADRA2A*) in impulsive decision-making in general and in DD in particular. Studies performed in rats and in humans revealed that the NE reuptake inhibitor atomoxetine improved performance and decreased premature responding in stop-signal reaction time tasks (as an example for motor impulsivity) (Bari & Robbins, 2013, Robinson et al., 2008). An experiment in rhesus monkeys has shown that the *ADRA2A* agonist guanfacine selectively reduced the animals' preference to choose smaller more immediate rewards (over larger delayed rewards) without affecting their risk preference (Kim et al., 2012). While the presynaptic function of the *ADRA2A* is to inhibit NE transmitter release (suppression of NE release by negative feedback), it is also the most prevalent postsynaptic NE receptor in the prefrontal cortex (PFC) (Arnsten et al., 1996). Thus, it has been proposed that an activation

of ADRA2As in the PFC might be the mechanism by which guanfacine decrases DD (Kim et al., 2012). Moreover, several genetic studies support an association of the G-allele of the *ADRA2A* C-1291G (rs1800544) single nucleotide polymorphism (SNP) with attention deficit hyperactivity disorder (ADHD) (Park et al., 2005, Roman et al., 2006, Schmitz et al., 2006). Accordingly, ADHD has also been associated with a preference to choose immediate over delayed rewards (Noreika et al., 2013) as well as with an increased vulnerability for addictive disorders (Urcelay & Dalley, 2012). Moreover, the G-allele of the ADRA2A rs1800544 SNP has been associated with higher vascular reactivity to cold and psychosocial stress corroborating the functional relevance of this SNP (Kelsey et al., 2012).

Acute cocaine administration affects the NE system by inhibiting NE reuptake at the respective transporters and thus increasing synaptic NE signaling (Clark et al., 1991, Fitzgerald, 2013, Levy & Blattberg, 1978, O'Neill et al., 2013). However, it is less clear how chronic cocaine use affects NE signaling. Recent evidence suggests that chronic use modifies the NE system by mechanisms involving ADRA2As (Fitzgerald, 2013). For example, rats exposed to seven days of cocaine injections displayed permanent desensitization of ADRA2As (measured by a blunted growth hormone response to clonidine challenges) (Baumann et al., 2004). Furthermore, the above mentioned *ADRA2A* agonist guanfacine has been associated with lower cocaine craving, anxiety, and arousal in patients recovering from cocaine dependence, whereas the *ADRA2A* antagonist yohimbine facilitated reinstatement of cocaine seeking during early withdrawal in rats (Buffalari et al., 2012, Fox et al., 2012).

Taken together, the NE system and in particular the *ADRA2A* are implicated in both DD and in the neurochemical adaptations after chronic cocaine consumption. Thus, our goal was to investigate whether three *ADRA2A* SNPs are associated with DD in chronic cocaine users compared to stimulant-naïve healthy controls. In addition to the afore-mentioned rs1800544, we further examined *ADRA2A* rs521674 and rs602618, which have been shown to be in linkage disequilibrium (LD) with rs1800544 in European and Asian populations (Clarke et al., 2012, Li et al., 2012). As specific radioligands for molecular imaging of the *ADRA2A* are still lacking, we determined ADRA2A mRNA expression levels in peripheral blood in order to clarify the functional significance of the investigated polymorphisms. We hypothesized that both the *ADRA2A* polymorphisms and the *ADRA2A* mRNA expression levels are associated with DD preferences in chronic cocaine users.

4.2 Methods

Participants

A total sample of 223 participants (129 chronic cocaine users and 94 stimulant-naïve controls) was investigated as part of the longitudinal Zurich Cocaine Cognition Study (ZuCo2St: Hulka et al., 2014, Vonmoos et al., 2013, Vonmoos et al., 2014). Cocaine users were included if they indicated cocaine as their primary drug of choice, showed a use of >0.5g/month, and a maximum abstinence duration no longer than 6 months. Exclusion criteria for all participants were any current or previous neurological disorders or head injuries and use of prescription drugs affecting cognitive functioning. Additional exclusion criteria for the cocaine users were regular use of opioids or a polytoxic drug use pattern according to DSM-IV as well as any current or previous Axis-I DSM-IV psychiatric disorders with exception of cocaine and alcohol abuse/dependence, a history of depression, and ADHD. Specific exclusion criteria for the control group were regular drug use (lifetime >15 occasions) with the exception of nicotine dependence and occasional cannabis use and any current or previous Axis-I DSM-IV psychiatric disorder (American Psychiatric Association, 1994). Participant's self-reports regarding drug use were confirmed by toxicological analyses of urine and hair samples. Our sample of 129 cocaine users consisted of 94 regular recreational users (72.8%) and 35 dependent users (27.1%) according to DSM-IV. More detailed information about our sample and about selection, recruitment, and drug screening procedures have been presented in detail in our previous work (Hulka et al., 2014, Preller et al., 2013, Vonmoos et al., 2013). The presented study has been carried out in accordance with the Declaration of Helsinki and was approved by the Cantonal Ethics Committee of Zurich. All participants provided written informed consent prior to the study and were financially compensated for their participation.

Procedure

All participants were examined by trained psychologists using a Structural Clinical Interview (SCID-I) (Wittchen et al., 1997) according to DSM-IV. Drug use was assessed by using a structured and standardized Interview for Psychotropic Drug Consumption (Quednow et al., 2004). The pre-morbid verbal intelligence quotient (IQ) was measured by the Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B; multiple choice vocabulary intelligence test) (Lehrl, 1999). Because psychiatric comorbidities such as depression and ADHD are common among cocaine using populations (Perez de Los Cobos et al., 2011, Rounsaville, 2004), we applied the ADHD-Self Rating Scale (ADHD-SR) (Rosler et al., 2004) and the Beck Depression Inventory (BDI) (Beck et al., 1961). In the frame of a comprehensive neuropsychological test battery, participants

completed a computerized version of a DD paradigm of the Kirby Monetary Choice Questionnaire *(Kirby & Petry, 2004)* using hypothetical rewards and fixed choices and enabling the calculation of the discounting rate of delayed rewards according to the formula $V=A/(1+ kD)$. Hereby, V is the present value of the delayed reward A at delay D, and k is a free parameter that determines the discounting rate (the larger the parameter k, the stronger the discounting of delayed rewards) (Mazur, 1987). In order to better illustrate comparisons between cocaine users and controls, the main parameter k was z-transformed (based on mean and standard deviation of the control group) and multiplied by -1 so that higher z-scores indicate lower levels of reward impulsivity (i.e., little discounting of delayed rewards).

Genotyping

The *ADRA2A* polymorphisms *(*rs1800544, rs521674, and rs602618) were determined using DNA extracted either from EDTA anticoagulated blood samples or from immortalized lymphoblastoids cell cultures after transforming the lymphocytes with Epstein-Barr virus. The isolation of the DNA followed the QIAGEN protocol for the Blood & Cell Culture DNA Isolation Maxi Kit (QIAGEN, Hilden, Germany). For PCR, we added 5µl of buffer containing the Universal PCR MasterMix (No AmpErase UNG) and the SNP Genotyping Assay (both provided by Applied Biosystems, Foster City, CA, USA) to 12.5ng air-dried DNA. PCR was performed according to the SNP Genotyping protocol supplied by Applied Biosystems. Both alleles were scored in a single well by measuring the fluorescence at the end of the PCR using a Tecan Ultra 384 reader (Tecan, Crailsheim, Germany). Excitation- and emission-wavelengths were 485 and 535 nm for the FAM-labeled probes and 535 and 590 nm for the VIC-labeled probes, respectively.

In order to compare G-allele carriers in rs1800544 with non-carriers, the rare GG genotype (8.8%) was pooled with the CG genotype resulting in two genotype groups: CC vs. CG/GG. Similarly, the rare TT genotype (8.8%) of rs521674 was pooled with the AT genotype and the rare CC genotype (9.2%) of rs602618 was pooled with the AC genotype, respectively, resulting in the two genotype groups: AA vs. AT/TT and AA vs. AC/CC. For the haplotype analysis (provided in the SI) of the three SNPs (rs1800544, rs521674, and rs602618) contrasting non-carrier vs. carriers of minor alleles (CC-AA-AA vs. CG/GG-AT/TT-AC/CC), ten participants with rare allele combinations were excluded.

mRNA Quantification

Total RNA was isolated from whole blood using the RNA isolation NucleoSpin RNA Blood in combination with the NucleoSpin RNA/DNA Buffer Set according to the manufacturer's recommendation (Macherey-Nagel AG, Oersingen, Switzerland). Total RNA samples were spectrophotometrically scanned (NanoVue, GE Healthcare Life Sciences) to obtain the A260/A280 of >1.9 and concentration levels. Additionally, for RNA quality resulting with RIN (RNA integrity number) values, all samples were measured on the automated electrophoresis system (Experion, BioRad Co., Hercules, CA, USA). Quantitative real-time RT-PCR was conducted for *ADRA2A* and six additional reference genes (*ACTB, GAPDH, ALAS1, RPL13A, PPIA,* and 18S ribosomal RNA) as described previously (Grunblatt et al., 2009). Total RNA (500 ng) from each sample was reverse transcribed using iScript cDNA synthesis kit (BioRad Co., Hercules, CA, USA). Each amplification was performed in a total volume of 20µl containing 5µl QuantiTect SYBR Green PCR kit (Qiagen) and the specific primer mix (PrimerAssay- Qiagen). PCR conditions were run according to manufacturer's manual (Qiagen). A melting point analysis was conducted for each assay to confirm specificity of PCR products and all PCR reactions were run in triplicates. The program LinRegPCR (www.hartfaalcentrum.nl) was used to determine the PCR efficiency. Gene expression and normalization analysis with the most stable reference genes was conducted using the QBase plus software (Biogazelle) (Vandesompele et al., 2002). The software detected that the reference gene *RPL13A* was least stable and therefore this gene was excluded and normalization analysis was conducted using the five other reference genes.

Statistical Analyses

Gene effects on DD were analyzed separately for each SNP using analyses of covariance (ANCOVA) with the factors *group* (twofold, cocaine users vs. controls) and *genotype* (twofold, CC vs. CG/GG) and the covariates *age* and *verbal IQ*. Another ANCOVA with the additional covariates *BDI score, ADHD-SR score,* and *cumulative cocaine dose* was calculated to investigate possible influences of these additional factors. *ADRA2A mRNA expression levels* were stratified into high vs. low mRNA expression using median split. DD was then investigated using ANCOVAs with the factors *group* (as above) and *mRNA expression levels* (high vs. low) and the covariates *age* and *verbal IQ*. Finally, *post hoc t*-tests were conducted on the basis of significant ANCOVA main effects where necessary. Results were considered significant if $p<.05$ after correction for multiple comparisons using the Hochberg method. Hochberg's procedure (a step-up modification of the Bonferroni method) tests each partition hypothesis using all the order statistics by

formulating a sequence of critical values based on Simes' inequality (Hochberg, 1988). Statistical analyses were conducted with SPSS (Version 20.0), testing of linkage disequilibrium (LD) between polymorphisms was performed with the software *Haploview* (Barrett et al., 2005) and associations between polymorphisms and cocaine use was analyzed with the Armitage's Trend Test.

4.3 Results

Demography and association analysis

The comparison between cocaine users and controls did not reveal significant differences regarding age and sex distribution (Table 7). However, cocaine users had significantly fewer years of education, lower IQ scores, higher scores in the BDI, and higher ADHD-SR scores than controls. Beyond these group differences, one-way ANCOVAs with age and IQ as covariates showed no significant *genotype* (rs1800544) or *group*genotype* interaction effects on BDI (genotype: $F(1,215)=0.40$, $p=.53$; interaction: $F(1,215)=0.03$, $p=.87$) or ADHD-SR scores (genotype: $F(1,215)=0.82$, $p=.37$; interaction: $F(1,215)=0.03$, $p=.85$).

Table 7. Demographic data of healthy controls and cocaine users (with exception of sex, means and *SD* are shown).

Demographic Data	**Controls (*n*=94)**	**Cocaine users (*n*=129)**	***t-test/X²***	***df***	***p***
Age, mean (*SD*)	30.0 (8.8)	30.0 (8.8)	-0.00	221	0.968
Sex (male / female)	68 / 26	94 / 35	0.0	1	0.524
Years of Education, mean (*SD*)	10.7 (1.8)	10.2 (1.7)	2.1	221	**0.038**
Verbal IQ, mean (*SD*)	107.1 (12.1)	102.4 (10.5)	3.0	183.1	**0.003**
BDI Score, mean (*SD*)	4.2 (4.0)	8.5 (7.0)	-5.8	210.2	**0.000**
ADHD-SR Score, mean (*SD*)	7.9 (5.0)	14.0 (9.3)	-6.2	204.0	**0.000**

ADHD-SR: Attention Deficit Hyperactivity Disorder-Self Rating Scale, BDI: Beck Depression Inventory, IQ: intelligence quotient

For information on the substance use of both groups see Table 8. Genotype frequencies of all three polymorphisms were distributed in accordance to the Hardy-Weinberg Equilibrium and all three SNPs were found to be in near perfect linkage disequilibrium (LD) with each other (rs1800544/rs521674: D'=1.0, LOD=92.24, $r^2=0.99$; rs1800544/rs602618: D'=0.98, LOD=79.62, $r^2=0.92$; rs521674/rs602618: D'=0.97,

LOD=77.66, r^2=0.91). None of the polymorphisms was associated with cocaine use per se and within the group of cocaine users, genotype groups did not differ regarding cocaine consumption.

Table 8. Substance use across groups.

Substance use	**Controls (*n*=94)**	**Cocaine users (*n*=129)**
Weekly[a] nicotine use, cigarettes, mean (*SD*)	62.0 (66.5)	90.4 (75.0)
Weekly[a] alcohol dose, g, mean (*SD*)	123.6 (135.9)	182.8 (194.2)
Weekly[a] cannabis use, g, mean (*SD*)	0.6 (1.4)	1.0 (2.8)
Weekly[a] MDMA use, g, mean (*SD*)	0.0 (0.0)	0.2 (0.9)
Weekly[a] amphetamine use, g, mean (*SD*)	0.0 (0.0)	0.1 (0.2)
Weekly[a] cocaine dose, g, mean (*SD*)	0.0 (0.0)	2.4 (5.0)
Years of cocaine use, mean (*SD*)	0.0 (0.0)	7.0 (5.5)
Cocaine concentration in hair, pg/mg, mean (*SD*)	0.0 (0.0)	7989 (18722)
Cumulative cocaine dose, g, mean (*SD*)	0.0 (0.0)	2143 (5798)
Self-reported cocaine abstinence duration, hours, mean (SD)	-	650.3 (861.5)
Recent cocaine use, urine samples, positive/negative/missing	0/94/0	26/102/1

[a]Weekly use is referring to the last six months, MDMA = 3,4-Methylendioxy-N-methylamphetamine

ADRA2A polymorphisms and DD

Results are reported for rs1800544, however, the same results have been found for the two other SNPs due to the strong linkage disequilibrium. As shown previously in a part of the present sample (Hulka et al., 2014), cocaine users were more likely to choose immediate smaller rewards over larger delayed rewards as indicated by significant *group* effects on DD in small, medium, and large reward magnitudes, respectively (Table 9). There was no significant main effect of the factor *genotype* but a significant *group*genotype* interaction was found across all reward magnitudes (Table 9), reflecting significantly

steeper DD in cocaine users carrying the G-allele (rs1800544, across all rewards: $F(1,215)=10.05$, $p_{cor}<.01$, Cohen's $f=0.22$) compared to CG/GG carriers (post hoc test of mean DD across reward magnitudes: $t(127)=2.93$, $p<.01$, $d=0.56$, Figure 8). In contrast, controls showed the reverse pattern but the post hoc test did not show a significant difference ($t(90)=-1.45$, $p=.15$, $d=0.33$). This interaction remained significant even when *BDI score, ADHD-SR score,* and *cumulative cocaine dose* were introduced together as additional covariates (rs1800544: $F(1,211)=8.93$, $p_{cor}<.01$, $\eta p^2=0.04$, Cohen's $f=0.21$). Of the covariates' main effects, none was strong enough to survive multiple comparison correction (age: $p_{cor}=.06$; verbal IQ: $p_{cor}=.57$; BDI score: $p_{cor}=.10$; ADHD-SR: $p_{cor}=.98$; cumulative cocaine dose: $p_{cor}=.08$).

Table 9. Analyses of covariance for delay discounting (DD) across reward magnitudes (corrected for *age* and *verbal IQ*).

	***ADRA2A* (rs1800544)**				
	F	*df*	p_{ncor}	ηp^2	p_{cor}
DD (across all rewards)					
Group	3.32	1	.070	.015	
Genotype	0.92	1	.338	.004	
Group*Genotype	10.05	1	**.002**	.045	**.004**
DD (small rewards)					
Group	7.89	1	**.005**	.035	**.010**
Genotype	2.14	1	.145	.010	
Group*Genotype	12.49	1	**.001**	.055	**.002**
DD (medium rewards)					
Group	8.05	1	**.005**	.036	**.005**
Genotype	2.07	1	.152	.010	
Group*Genotype	7.27	1	**.008**	.033	**.008**
DD (large rewards)					
Group	6.55	1	**.011**	.030	**.033**
Genotype	1.96	1	.163	.009	
Group*Genotype	6.98	1	**.009**	.031	**.027**

DD = delay discounting, p_{ncor}: uncorrected *p*-values, p_{cor}: p-values corrected for multiple comparisons

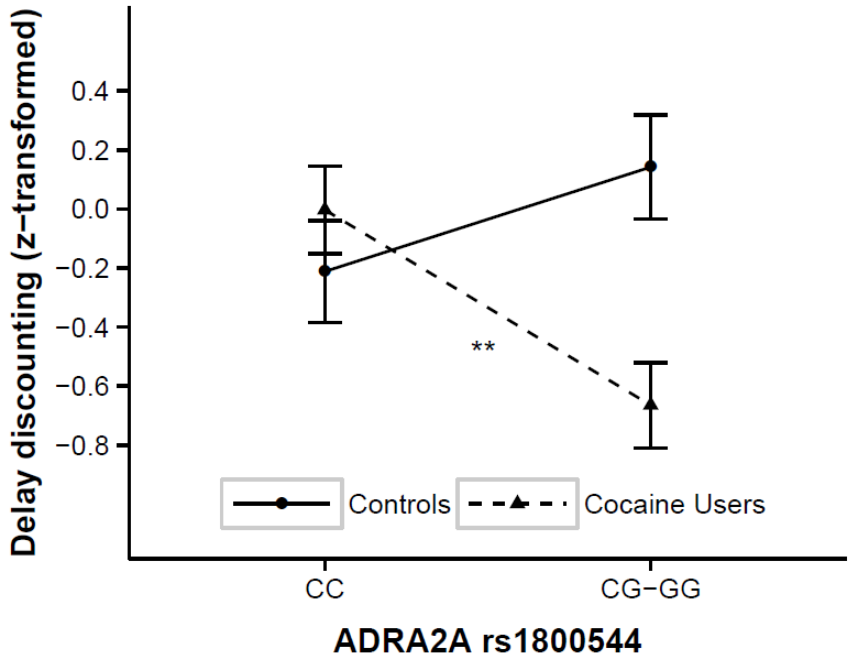


Figure 8. Mean delay discounting (DD) across reward magnitudes for rs1800544 with significant (p<0.01) *group*genotype* interactions (mean and *SE*, z-transformed and corrected for *age* and *verbal IQ*). Higher scores depict decreased discounting, whereas lower scores show increased discounting. Significant post hoc t-test on genotype (CC vs. CG-GG) in cocaine users: **p<.01.

ADRA2A mRNA expression and DD

After mRNA quality control, 104 samples were eligible for the *ADRA2A* gene expression analysis. Peripheral mRNA expression did not differ between cocaine users (n=54) and controls (n=50) and between genotype groups. However, using a median split of the mRNA expression (high vs. low) together with the factor *group* in a two-way ANCOVA revealed a significant *group*mRNA expression* interaction on mean DD across rewards ($F(1,98)$=5.22, p<.05, ηp^2=0.05, Cohen's f=0.23, Figure 9), mirroring the *group*genotype* interaction shown above (Figure 8). The interaction was explained by more pronounced DD in cocaine users with low levels of *ADRA2A* mRNA expression compared to users with high levels, as indicated by a strong statistical trend and a considerable effect size in the post hoc test (t(52)=1.83, p=.07, d=0.50). Again, controls showed the reverse pattern, which was not significant (t(48)=-1.53, p=.13, d=.43). Importantly, within the cocaine user group, the period of abstinence of cocaine use (see Table 7) was neither correlated with *ADRA2A* mRNA expression (r=-.12, p=.36, n=54) nor with DD (r=.05, p=.59, n=129). Also, a positive urine test for cocaine (see Table 7) did not affect *ADRA2A* mRNA expression level in cocaine users

(negative: 1.33+/-1.0 *SD*; positive 1.19+/-0.8 *SD*; *t*(51)=.44, *p*=.66). Moreover, we have previously shown in an overlapping sample, that a cocaine positive urine test does not affect DD (Hulka et al., 2014). Accordingly, inclusion of abstinence duration or cocaine urine screening status as additional covariates in the analyses did not change our results.

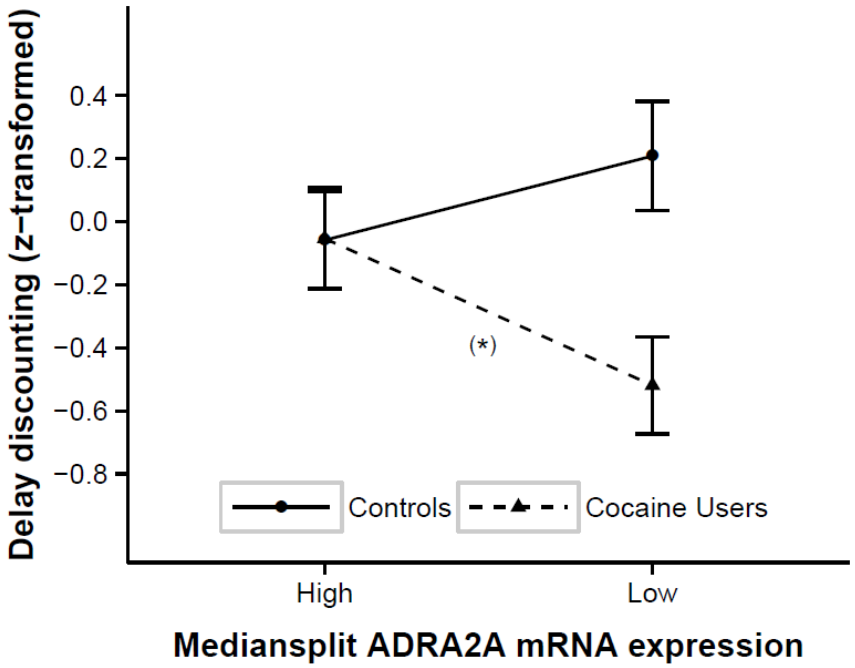


Figure 9. Mean delay discounting (DD) across reward magnitudes in cocaine users and controls stratified for high vs. low *ADRA2A* mRNA expression levels (mean and *SE*, z-transformed and corrected for *age* and *verbal IQ*). Higher scores depict decreased discounting, whereas lower scores show increased discounting. Near-significant post hoc t-test on genotype (CC vs. CG-GG) in cocaine users: $^{(*)}p$=.07.

4.4 Discussion

Examining the molecular mechanisms of DD in cocaine users and healthy controls, we were able to demonstrate that three linked *ADRA2A* SNPs (rs1800544, rs521675, and rs602618) as well as peripheral *ADRA2A* mRNA expression levels mediate reward impulsivity in dependence of cocaine use of the participants. More specifically, we found that in cocaine users, the G-allele of rs1800544 (and the T-allele of rs521674, as well as the C-allele of rs602618) as well as low *ADRA2A* mRNA concentrations were associated with steeper discounting of delayed rewards, while there were no significant differences between genotypes in healthy controls.

Interestingly, the current findings are in line with previous reports on the role of *ADRA2A* polymorphisms in ADHD, as the G-allele of rs1800544 has repeatedly been proposed as a risk allele for ADHD as well as for its symptoms of impulsive decision-making (Gizer et al., 2009, Park et al., 2005, Roman et al., 2006, Roman et al., 2003, Schmitz et al., 2006, Stevenson et al., 2005). However, a large meta-analysis found no consistent evidence for a relationship between the G-allele and ADHD but a significant heterogeneity of results from different studies (Gizer et al., 2009). Consequently, the authors suggested that future studies should explore potential moderators as explanation for this heterogeneity. Accordingly, a recent study on the relationship between *ADRA2A* SNPs (rs1800544, rs553668, and rs1800545) and ADHD found mediating effects of personality (namely associations with novelty seeking, persistence, and harm avoidance) (de Cerqueira et al., 2011). In addition, another study presenting an association of rs1800544 with intra-individual variability in response time suggested that *ADRA2A* SNPs are rather associated with objectively measureable endophenotypes of ADHD (such as intra-individual variability in response time) than with the complete clinical phenotype of ADHD itself (Cummins et al., 2014). In line with these two studies, we provide evidence for an interaction between *ADRA2A* SNPs and cocaine use regarding DD, which has been proposed as an addiction endophenotype (MacKillop, 2013).

Beyond its association with ADHD symptoms, the G-allele of rs1800544 has been found to be related to alcohol dependence and tobacco smoking (Karahalil et al., 2008, Prestes et al., 2007). In addition, it has been discovered that carrying the G-allele was associated with a higher consumption of sweets (such as chocolate, candies and nougat) in a study with children and adolescents (Maestu et al., 2007) and also with an elevated stress response to cold and psychosocial stressors in adolescents and young adults (Kelsey et al., 2012). Finally, even though only few studies focused on the rs521674 and rs602618 polymorphisms, they have recently been associated with a positive family history of alcoholism (Clarke et al., 2012). These findings support the view that the minor alleles of these SNPs might impact addictive behaviors given that the common denominator of alcohol and tobacco dependence, stimulant addiction, and obesity is the

propensity to favor immediate rewards at the expense of negative future consequence (MacKillop, 2013, Mazur, 1987). However, it should be noted that, beyond the shown *group*genotype* interaction on DD, we did not find an association between the investigated *ADRA2A* SNPs and cocaine use per se.

It is still uncertain whether the three investigated SNPs mapping to the promoter region of the *ADRA2A* gene have functional significance. However, theoretical transcription binding sites suggest that at least rs521674 and rs60218 create new transcription binding sites at the T and C polymorphisms, respectively (e.g., http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3). Additionally, the mRNA quantification in our present study provides an additional source of information. We found that cocaine users with high peripheral *ADRA2A* mRNA expression levels showed normal DD, while cocaine users with low mRNA expression levels revealed a strong DD endophenotype even though this effect was only a statistical trend (p=.07) in the post hoc comparison (Figure 2). Notably, the sample size for the mRNA expression analysis was much lower than for the SNP analysis (only 104 participants were eligible for the mRNA expression analysis) due to our strict mRNA quality control and, thus, the gene expression analysis was less well powered. However, post hoc power analyses revealed that group*genotype (f=0.22) and group*gene expression (f=0.23) interactions on DD displayed highly similar effect sizes. Moreover, also the post hoc comparisons on DD in the cocaine group showed comparable effect sizes in the genotype (G vs. CG/GG: d=0.56) and gene expression analysis (high vs low: d=0.50). With this, also our mRNA findings corroborate that cocaine use moderates the effects of *ADRA2A* genotype on DD. Support for this assumption stems from two animal studies. On the one hand, chronic cocaine administration has been shown to desensitize or downregulate postsynaptic ADRA2As from 42 hours on up to eight days later in the rat brain (Baumann et al., 2004). On the other hand, the *ADRA2A* agonist guanfacine was able to reduce impulsivity in rhesus monkeys by increasing the proportion of choices of larger delayed rewards over smaller immediate rewards (Kim et al., 2012). Based on these two studies, we postulate that control individuals have unaffected ADRA2As, whereas in cocaine users, ADRA2As may have been desensitized or downregulated by chronic drug consumption. Under these circumstances, higher receptor mRNA expression may be capable of mimicking the beneficial effects of guanfacine resulting in normal DD.

In line with our findings, a recent review highlights the potential of guanfacine as a therapeutic agent to attenuate stress- and cue-induced craving in cocaine-dependent individuals (Fox & Sinha, 2014). The authors further propose that guanfacine may be an effective medication to reduce craving and relapse vulnerability in other forms of addiction as well, due to its ability to improve cognitive and emotional control over drug-seeking behavior (Fox & Sinha, 2014).

The present study has two limitations. First, because of the low frequency of the GG genotype of rs1800544 (8.8%) in combination with our limited sample size, we decided to contrast G-allele carriers against non-carriers. Even though the threefold genotype comparison (CC vs. CG vs. GG) revealed comparable results, we have chosen to rely on the two-group comparisons for our interpretations. Second, because of the cross-sectional analysis in this study, it is not possible to answer conclusively whether the *ADRA2A* genotype*cocaine use interaction on DD develops as a consequence of cocaine use or if DD together with *ADRA2A* SNPs predispose cocaine addiction, as there is evidence for both interpretations (Brody et al., 2014). Our recent longitudinal results suggest that – in contrast to self-reported impulsivity and decision-making in the Iowa Gambling Task – DD is largely stable in cocaine users and does not change with decreased or increased use supporting the view that DD might be a predisposition and a potential endophenotype of cocaine addiction (Hulka et al., 2015). However, the sample size of our longitudinal study is too small to investigate potential genotype effects on the course of DD. Thus, to draw definite conclusions, future studies should use longitudinal designs with larger samples in order to investigate the causal relationships between DD, cocaine use, and *ADRA2A* SNPs more in depth.

In conclusion, we presented evidence that relationship between the DD endophenotype and cocaine use is moderated by three SNPs of the *ADRA2A* gene as well as by *ADRA2A* gene expression suggesting that the norepinephrine system is involved in DD observed in cocaine users. As DD deficits may underlie addictive disorders in general, our results suggest that pharmacological compounds targeting *ADRA2A* (such as guanfacine) might be considered as option for symptom-specific treatment of DD in stimulant addiction.

5 Overall conclusion

The aim of this PhD project was to investigate different aspects of emotion regulation deficits (psychological, pharmacological, and genetic) across several psychiatric disorders using psychophysiological, neurobiological, and genetic methods. Based on the diathesis-stress model, we hypothesized that specific environmental factors in interaction with individual predispositions / vulnerabilities lead to specifically measurable emotion regulation deficits.

Previous research on emotion regulation has almost exclusively focused on certain emotion regulation strategies, such as reappraisal and suppression (Berking & Wupperman, 2012). We, on the other hand, chose to investigate emotion regulation in a broader sense, examining specific examples of situations where emotion regulation occurs outside of the narrow research focus of reappraisal and suppression. However, our examples also fall perfectly within the definition of emotion regulation:

Emotions are coordinated response sets aiming to orchestrate the best possible reaction to an event that offers opportunities or challenges for an individual (Lang, 1995, Lazarus, 1993). Emotion regulation includes all conscious or unconscious processes by which individuals influence which negative or positive emotions they experience, how and when they experience them, and how they express them (Gross et al., 2006, Quoidbach et al., 2010, Thompson, 1994).

In study A, participants were confronted with stressful electrical shocks (i.e., a challenging situation) and were compelled to manage their anxiety response (i.e., their emotional reaction). In study B, participants were administered GHB and instructed to manage their sexual arousal, and in study C, cocaine users were compared to healthy controls concerning their ability to delay gratification.

The diathesis-stress model postulates that psychiatric disorders develop if environmental factors exceed a coping threshold defined by an individual's predispositions (Lazarus, 1993). Our studies provided evidence that this principle holds true for the formation of emotion regulation deficits. Study A showed that when participants were confronted with uncontrollable and unpredictable stressors (i.e., the environmental factor), the management of anxiety depended on participants' predispositions for anxiety disorders and depression (i.e., the individual factor). Consistently, study C revealed that cocaine consumption (i.e., the environmental factor) interacted with genetic predispositions for impulsivity (i.e., the individual factor) on their ability to delay gratification. In study B, we did not find an individual factor influencing our results. This might be due to the very homogenous sample we deliberately chose to compare our neuroimaging results with previous research. However, it can be expected that future studies will find individually differing response rates to GHB.

In addition to the model of emotion regulation and the diathesis-stress model, we utilized other psychopathological models in our studies. In study A, we used the learned helplessness model and the safety signal hypothesis, and in study C, we used the proposed delay discounting endophenotype for cocaine addiction. Thereby, this PhD project further illustrates an important fact in psychological or psychopathological research: There is an abundance of diverse models and hypotheses applying general principles to explain specific disorders. For instance, the learned helplessness model could be seen as an example of the diathesis-stress model describing the development of a specific form of emotion regulation deficit (namely depression). Because of that, there is often conceptual overlap between different models, and there is, in our opinion, an increasing need to integrate and unite these varying models. As such, the methodological approach of this PhD project fits within the framework of RDoC (research domain criteria, NIMH.gov), which proposes not to consider diagnostic entities, but rather functional domains, as research targets of disease mechanism understanding and target identification of therapy development.

Thus, we propose that applying the diathesis-stress model to the development of emotion regulation deficits could be useful as an encompassing psychopathological model for many psychiatric disorders. In this PhD project, we focused on emotion regulation deficits in anxiety disorders, depression, and substance disorders. However, emotion regulation deficits are important in many additional psychiatric conditions, including personality disorders, eating disorders, and somatoform disorders, just to name a few (Berking & Wupperman, 2012). Thus, investigating emotion regulation deficits in psychiatric disorders might even prove useful to explain the frequent comorbidities of psychiatric disorders in general, and of anxiety disorders, depression, and substance disorders in particular (Myrick & Brady, 2003).

In conclusion, we characterized various aspects of specific emotion regulation deficits in the three studies of this PhD project. As a result, we provided evidence that the diathesis-stress model can be applied to explain the formation of emotion regulation deficits in psychiatric conditions. Now, future research should investigate whether this approach is useful as an encompassing model for diverse psychiatric disorders. With certainty, it is important that future research proceeds to integrate and unite different models from different research traditions.

6 Addendum: Summaries of the additional publications

In addition to the three main studies, two further publications arose out of this PhD project. They are different from the main studies in terms of content but for the sake of completeness, they will be briefly summarized here as well. The following summaries are extracted from:

- Havranek MM, Volkart F, Bolliger B, Roos S, Buschner M, Mansour R, Chmielewski T, Gaudlitz K, Hättenschwiler J, Seifritz E, Ruch W. The fear of being laughed at as a diagnostic criterion in social anxiety disorder and avoidant personality disorder. In submission.

  and

- Havranek MM, Vonmoos M, Müller CP, Büetiger JR, Tasiudi E, Hulka LM, Preller KH, Mössner R, Grünblatt E, Seifritz E, Quednow BB. Serotonin transporter and tryptophan hydroxylase gene variations mediate working memory deficits of cocaine users. Neuropsychopharmacology. 2015. doi: 10.1038/npp.2015.146.

The fear of being laughed at as a diagnostic criterion in social anxiety disorder and avoidant personality disorder

Background: Social anxiety disorder (SAD) is the most common anxiety disorder and has a considerable negative impact on social functioning, quality of life and career progression of those affected. Gelotophobia, the fear of being laughed at, shares many similarities, and has therefore been proposed as a subtype of SAD.

Methods: Here, we investigated the relationship between SAD and gelotophobia by examining a sample of 133 participants (64 psychiatric patients and 69 healthy controls matched for age and sex) using the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders (4th edition) and two separate rating instruments for gelotophobia (GELOPH<15>, Picture-Geloph).

Results: As expected, gelotophobia scores and the number of gelotophobic individuals was higher in patients compared to healthy controls. Additionally, occurrence was higher in patients with SAD and avoidant personality disorder (APD) compared to patients with other psychiatric disorders. Furthermore, gelotophobia scores were highly correlated with scores in SAD and APD, and although we also found gelotophobic individuals without SAD and APD, we found no patients suffering from both SAD and APD that were not gelotophobic.

Conclusions: Our data showed a large diagnostic overlap between gelotophobia and SAD but it did not suggest that gelotophobia is a subtype of SAD. The observed pattern rather implies that the fear of being laughed at may be compared to one of the specifically feared social or performance situations characteristic of SAD. As such, gelotophobic symptoms may prove valuable as an additional diagnostic criterion for SAD and APD.

Serotonin transporter and tryptophan hydroxylase gene variations mediate working memory deficits of cocaine users

Background: Cocaine users consistently develop working memory (WM) impairments but the mediating molecular mechanisms are unknown so far. Recent evidence suggests that the serotonin (5-HT) system is altered by chronic cocaine use, while also being involved in WM processing. Thus, we investigated the effects of genetic variations impacting 5-HT activity and of peripheral 5-HT transporter (5-HTT) mRNA expression on WM performance in cocaine users and stimulant-naïve controls.

Methods: 220 participants (126 cocaine users, 94 controls) were assessed with visuospatial, spatial and verbal WM tasks, genotyped for the length polymorphism in the promoter region of the 5-HTT (*5-HTTLPR*), the variable number of tandem repeats in the second intron of the 5-HTT (VNTR In2), two single nucleotide polymorphisms (rs4570625, rs1386497) in the tryptophan hydroxylase-2 (TPH2) gene, and quantified for peripheral 5-HTT mRNA expression in whole blood samples.

Results: Several significant gene*environment interactions between 5-HT genotypes and cocaine use on WM emerged: In cocaine users, the long/long (*5-HTTLPR)*, 9+10/9+10 (VNTR In2) and C/C (TPH2 rs1386497) genotypes were risk-alleles for WM impairments, while in healthy controls these polymorphisms were associated with improved WM performance. Analogously, high 5-HTT mRNA levels were associated with worse executive WM performance in cocaine users, but with increased performance in controls.

Conclusions: These gene*environment interactions suggest that the 5-HT system plays an important role in the development of cognitive deficits in chronic cocaine users. Hence, pharmacological compounds targeting 5-HT neurotransmission might be promising for the treatment of cognitive deficits in cocaine dependence.

7 References

Aldao A, Nolen-Hoeksema S. When are adaptive strategies most predictive of psychopathology? J Abnorm Psychol. 2012;121(1):276-81.

Aldao A, Nolen-Hoeksema S, Schweizer S. Emotion-regulation strategies across psychopathology: A meta-analytic review. Clin Psychol Rev. 2010;30(2):217-37.

Allen NB, Trinder J, Brennan C. Affective startle modulation in clinical depression: preliminary findings. Biol Psychiatry. 1999;46(4):542-50.

Amat J, Baratta MV, Paul E, Bland ST, Watkins LR, Maier SF. Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. Nat Neurosci. 2005;8(3):365-71.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (4th edn) (DSM-IV). Washington, DC: APA; 1994.

Andresen H, Aydin BE, Mueller A, Iwersen-Bergmann S. An overview of gamma-hydroxybutyric acid: pharmacodynamics, pharmacokinetics, toxic effects, addiction, analytical methods, and interpretation of results. Drug Test Anal. 2011;3(9):560-8.

Arnsten AF, Steere JC, Hunt RD. The contribution of alpha 2-noradrenergic mechanisms of prefrontal cortical cognitive function. Potential significance for attention-deficit hyperactivity disorder. Arch Gen Psychiatry. 1996;53(5):448-55.

Azzinnari D, Sigrist H, Staehli S, Palme R, Hildebrandt T, Leparc G, Hengerer B, Seifritz E, Pryce CR. Mouse social stress induces increased fear conditioning, helplessness and fatigue to physical challenge together with markers of altered immune and dopamine function. Neuropharmacology. 2014;85:328-41.

Bach DR, Friston KJ, Dolan RJ. Analytic measures for quantification of arousal from spontaneous skin conductance fluctuations. Int J Psychophysiol. 2010;76(1):52-5.

Baker SR, Stephenson D. Prediction and control as determinants of behavioural uncertainty: effects on task performance and heart rate reactivity. Integr Physiol Behav Sci. 2000;35(4):235-50.

Baker TB, Piper ME, McCarthy DE, Majeskie MR, Fiore MC. Addiction Motivation Reformulated: An Affective Processing Model of Negative Reinforcement. Psychol Rev. 2004;111(1):33-51.

Bari A, Robbins TW. Noradrenergic versus dopaminergic modulation of impulsivity, attention and monitoring behaviour in rats performing the stop-signal task: possible relevance to ADHD. Psychopharmacology (Berl). 2013;230(1):89-111.

Barreiro LB, Laval G, Quach H, Patin E, Quintana-Murci L. Natural selection has driven population differentiation in modern humans. Nat Genet. 2008;40(3):340-5.

Barrett J, Armony JL. Influence of trait anxiety on brain activity during the acquisition and extinction of aversive conditioning. Psychol Med. 2009;39(2):255-65.

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21(2):263-5.

Baumann MH, Milchanowski AB, Rothman RB. Evidence for alterations in alpha2-adrenergic receptor sensitivity in rats exposed to repeated cocaine administration. Neuroscience. 2004;125(3):683-90.

Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. J Consult Clin Psychol. 1988;56(6):893-7.

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961;4:561-71.

Beddington J, Cooper CL, Field J, Goswami U, Huppert FA, Jenkins R, Jones HS, Kirkwood TB, Sahakian BJ, Thomas SM. The mental wealth of nations. Nature. 2008;455(7216):1057-60.

Benavides J, Rumigny JF, Bourguignon JJ, Cash C, Wermuth CG, Mandel P, Vincendon G, Maitre M. High affinity binding sites for gamma-hydroxybutyric acid in rat brain. Life sciences. 1982;30(11):953-61.

Berking M, Margraf M, Ebert D, Wupperman P, Hofmann SG, Junghanns K. Deficits in emotion-regulation skills predict alcohol use during and after cognitive–behavioral therapy for alcohol dependence. J Cons Clin Psychol. 2011;79(3):307-18.

Berking M, Orth U, Wupperman P, Meier LL, Caspar F. Prospective effects of emotion-regulation skills on emotional adjustment. J Couns Psychol. 2008;55(4):485-94.

Berking M, Wupperman P. Emotion regulation and mental health: Recent finding, current challenges, and future directions. Curr Opin Psychiatry. 2012;25(2):128-34.

Bessman SP, Fishbein WN. Gamma-Hydroxybutyrate, a Normal Brain Metabolite. Nature. 1963;200:1207-8.

Bickel WK, Landes RD, Christensen DR, Jackson L, Jones BA, Kurth-Nelson Z, Redish AD. Single- and cross-commodity discounting among cocaine addicts: the commodity and its temporal location determine discounting rate. Psychopharmacology (Berl). 2011;217(2):177-87.

Bosch OG, Eisenegger C, Gertsch J, von Rotz R, Dornbierer D, Gachet MS, Heinrichs M, Wetter TC, Seifritz E, Quednow BB. Gamma-hydroxybutyrate enhances mood and prosocial behavior without affecting plasma oxytocin and testosterone. Psychoneuroendocrinology. 2015;62:1-10.

Bosch OG, Quednow BB, Seifritz E, Wetter TC. Reconsidering GHB: orphan drug or new model antidepressant? Journal of psychopharmacology. 2012;26(5):618-28.

Boucsein W. Electrodermal activity (2nd ed.). New York, NY, US: Springer Science + Business Media; 2012.

Bradley BP, Mogg K, White J, Groom C, de Bono J. Attentional bias for emotional faces in generalized anxiety disorder. Br J Clin Psychol. 1999;38 (Pt 3):267-78.

Brancucci A, Berretta N, Mercuri NB, Francesconi W. Gamma-hydroxybutyrate and ethanol depress spontaneous excitatory postsynaptic currents in dopaminergic neurons of the substantia nigra. Brain research. 2004;997(1):62-6.

Breier A, Albus M, Pickar D, Zahn TP, Wolkowitz OM, Paul SM. Controllable and uncontrollable stress in humans: alterations in mood and neuroendocrine and psychophysiological function. A J Psychiatry. 1987;144(11):1419-25.

Brody GH, Yu T, MacKillop J, Miller GE, Chen E, Obasi EM, Beach SR. Catecholamine levels and delay discounting forecast drug use among African American youths. Addiction. 2014;109(7):1112-8.

Buffalari DM, Baldwin CK, See RE. Treatment of cocaine withdrawal anxiety with guanfacine: relationships to cocaine intake and reinstatement of cocaine seeking in rats. Psychopharmacology (Berl). 2012;223(2):179-90.

Campbell-Sills L, Ellard KK, Barlow DH. Emotion regulation in anxiety disorders. In: Gross JJ, Gross JJ, editors. Handbook of emotion regulation (2nd ed). New York, NY, US: Guilford Press; 2014. p. 393-412.

Canli T, Ferri J, Duman EA. Genetics of emotion regulation. Neuroscience. 2009;164(1):43-54.

Carlson NR. Physiology of behavior (5th ed.). Needham Heights, MA, US: Allyn & Bacon; 1994.

Carter LP, Koek W, France CP. Behavioral analyses of GHB: receptor mechanisms. Pharmacol Ther. 2009;121(1):100-14.

Chong TT, Cunnington R, Williams MA, Kanwisher N, Mattingley JB. fMRI adaptation reveals mirror neurons in human inferior parietal cortex. Curr Biol. 2008;18(20):1576-80.

Christianson JP, Benison AM, Jennings J, Sandsmark EK, Amat J, Kaufman RD, Baratta MV, Paul ED, Campeau S, Watkins LR, Barth DS, Maier SF. The sensory insular cortex mediates the stress-buffering effects of safety signals but not behavioral control. J Neurosci. 2008;28(50):13703-11.

Clark AJ, Butcher SP, Winn P. Evidence for functional separation of alpha-1 and alpha-2 noradrenaline receptors by pre-synaptic terminal re-uptake mechanisms. Psychopharmacology (Berl). 1991;103(3):366-74.

Clarke TK, Dempster E, Docherty SJ, Desrivieres S, Lourdsamy A, Wodarz N, Ridinger M, Maier W, Rietschel M, Schumann G. Multiple polymorphisms in genes of the adrenergic stress system confer vulnerability to alcohol abuse. Addict Biol. 2012;17(1):202-8.

Coffey SF, Gudleski GD, Saladin ME, Brady KT. Impulsivity and rapid discounting of delayed hypothetical rewards in cocaine-dependent individuals. Exp Clin Psychopharmacol. 2003;11(1):18-25.

Cooper RM, Langton SR. Attentional bias to angry faces using the dot-probe task? It depends when you look for it. Behav Res Ther. 2006;44(9):1321-9.

Cruz HG, Ivanova T, Lunn ML, Stoffel M, Slesinger PA, Luscher C. Bi-directional effects of GABA(B) receptor agonists on the mesolimbic dopamine system. Nat Neurosci. 2004;7(2):153-9.

Cummins TD, Jacoby O, Hawi Z, Nandam LS, Byrne MA, Kim BN, Wagner J, Chambers CD, Bellgrove MA. Alpha-2A adrenergic receptor gene variants are associated with increased intra-individual variability in response time. Mol Psychiatry. 2014;19(9):1031-6.

Davey GCL, Burgess I, Rashes R. Coping strategies and phobias: The relationship between fears, phobias and methods of coping with stressors. Br J Clin Psychol. 1995;34(3):423-34.

de Cerqueira CC, Polina ER, Contini V, Marques FZ, Grevet EH, Salgado CA, da Silva PO, Picon FA, Belmonte-de-Abreu P, Bau CH. ADRA2A polymorphisms and ADHD in adults: possible mediating effect of personality. Psychiatry Res. 2011;186(2-3):345-50.

Diener C, Kuehner C, Brusniak W, Struve M, Flor H. Effects of stressor controllability on psychophysiological, cognitive and behavioural responses in patients with major depression and dysthymia. Psychol Med. 2009;39(1):77-86.

Diener C, Struve M, Balz N, Kuehner C, Flor H. Exposure to uncontrollable stress and the postimperative negative variation (PINV): prior control matters. Biol Psychol. 2009;80(2):189-95.

Ehring T, Fischer S, Schnülle J, Bösterling A, Tuschen-Caffier B. Characteristics of emotion regulation in recovered depressed versus never depressed individuals. Pers Indiv Dif. 2008;44(7):1574-84.

Ekman P, Friesen W. Pictures of facial affect. Palo Alto: Consulting Psychologists; 1975.

Engberg G, Nissbrandt H. gamma-Hydroxybutyric acid (GHBA) induces pacemaker activity and inhibition of substantia nigra dopamine neurons by activating GABAB-receptors. Naunyn Schmiedebergs Arch Pharmacol. 1993;348(5):491-7.

Fitzgerald PJ. Elevated norepinephrine may be an etiological factor in a wide range of diseases: age-related macular degeneration, systemic lupus erythematosus, atrial fibrillation, metabolic syndrome. Med Hypotheses. 2013;80(5):558-63.

Fox H, Sinha R. The role of guanfacine as a therapeutic agent to address stress-related pathophysiology in cocaine-dependent individuals. Adv Pharmacol. 2014;69:217-65.

Fox HC, Seo D, Tuit K, Hansen J, Kimmerling A, Morgan PT, Sinha R. Guanfacine effects on stress, drug craving and prefrontal activation in cocaine dependent individuals: preliminary findings. J Psychopharmacol. 2012;26(7):958-72.

Frohmader KS, Pitchers KK, Balfour ME, Coolen LM. Mixing pleasures: review of the effects of drugs on sex behavior in humans and animal models. Horm Behav. 2010;58(1):149-62.

Gamble SA, Conner KR, Talbot NL, Yu Q, Tu XM, Connors GJ. Effects of pretreatment and posttreatment depressive symptoms on alcohol consumption following treatment in Project MATCH. J Stud Alcohol Drugs. 2010;71(1):71-7.

Gizer IR, Ficks C, Waldman ID. Candidate gene studies of ADHD: a meta-analytic review. Hum Genet. 2009;126(1):51-90.

Glotzbach-Schoon E, Tadda R, Andreatta M, Troger C, Ewald H, Grillon C, Pauli P, Muhlberger A. Enhanced discrimination between threatening and safe contexts in high-anxious individuals. Biol Psychol. 2013;93(1):159-66.

Graf H, Walter M, Metzger CD, Abler B. Antidepressant-related sexual dysfunction - perspectives from neuroimaging. Pharmacol Biochem Behav. 2014;121:138-45.

Graf H, Wiegers M, Metzger CD, Walter M, Gron G, Abler B. Erotic stimulus processing under amisulpride and reboxetine: a placebo-controlled fMRI study in healthy subjects. Int J Neuropsychopharmacol. 2014;18(2).

Greenberg PE, Kessler RC, Birnbaum HG, Leong SA, Lowe SW, Berglund PA, Corey-Lisle PK. The economic burden of depression in the United States: how did it change between 1990 and 2000? J Clin Psychiatry. 2003;64(12):1465-75.

Grillon C. Startle reactivity and anxiety disorders: aversive conditioning, context, and neurobiology. Biol Psychiatry. 2002;52(10):958-75.

Grillon C, Baas JP, Lissek S, Smith K, Milstein J. Anxious responses to predictable and unpredictable aversive events. Behav Neurosci. 2004;118(5):916-24.

Grillon C, Lissek S, Rabin S, McDowell D, Dvir S, Pine DS. Increased anxiety during anticipation of unpredictable but not predictable aversive stimuli as a psychophysiologic marker of panic disorder. A J Psychiatry. 2008;165(7):898-904.

Grillon C, Pine DS, Lissek S, Rabin S, Bonne O, Vythilingam M. Increased anxiety during anticipation of unpredictable aversive stimuli in posttraumatic stress disorder but not in generalized anxiety disorder. Biol Psychiatry. 2009;66(1):47-53.

Gross JJ. The emerging field of emotion regulation: An integrative review. Rev Gen Psychol. 1998;2(3):271-99.

Gross JJ. Emotion regulation: Conceptual and empirical foundations. In: Gross JJ, editor. Handbook of emotion regulation. 2nd ed. New York, NY: Guilford Press; 2014. p. 3-20.

Gross JJ, Richards JM, John OP. Emotion regulation in everyday life. In: Snyder DK, Simpson JA, Hughes JN, editors. Emotion regulation in couples and families: Pathways to dysfunction and health Washington, DC: American Psychological Association; 2006. p. 13-35.

Gross JJ, Thompson RA. Emotion regulation: Conceptual foundations. In: Gross JJ, editor. Handbook of emotion regulation. New York, NY: Guilford Press; 2007. p. 3-24.

Grunblatt E, Bartl J, Zehetmayer S, Ringel TM, Bauer P, Riederer P, Jacob CP. Gene expression as peripheral biomarkers for sporadic Alzheimer's disease. J Alzheimers Dis. 2009;16(3):627-34.

Hagemann JH, Berding G, Bergh S, Sleep DJ, Knapp WH, Jonas U, Stief CG. Effects of visual sexual stimuli and apomorphine SL on cerebral activity in men with erectile dysfunction. European urology. 2003;43(4):412-20.

Hammen C. Stress and depression. Annu Rev Clin Psychol. 2005;1:293-319.

Hechler V, Gobaille S, Bourguignon JJ, Maitre M. Extracellular events induced by gamma-hydroxybutyrate in striatum: a microdialysis study. J Neurochem. 1991;56(3):938-44.

Hedou G, Chasserot-Golaz S, Kemmel V, Gobaille S, Roussel G, Artault JC, Andriamampandry C, Aunis D, Maitre M. Immunohistochemical studies of the localization of neurons containing the enzyme that synthesizes dopamine, GABA, or gamma-hydroxybutyrate in the rat substantia nigra and striatum. J Comp Neurol. 2000;426(4):549-60.

Heil SH, Johnson MW, Higgins ST, Bickel WK. Delay discounting in currently using and currently abstinent cocaine-dependent outpatients and non-drug-using matched controls. Addict Behav. 2006;31(7):1290-4.

Herry C, Bach DR, Esposito F, Di Salle F, Perrig WJ, Scheffler K, Luthi A, Seifritz E. Processing of temporal unpredictability in human and animal amygdala. J Neurosci. 2007;27(22):5958-66.

Hirschfeld RM. The Comorbidity of Major Depression and Anxiety Disorders: Recognition and Management in Primary Care. Prim Care Companion J Clin Psychiatry. 2001;3(6):244-54.

Hochberg Y. A sharper Bonferroni procedure for multiple tests of significance. Biometrika. 1988;75(4):3.

Holstege G, Georgiadis JR, Paans AM, Meiners LC, van der Graaf FH, Reinders AA. Brain activation during human male ejaculation. J Neurosci. 2003;23(27):9185-93.

Hulka LM, Eisenegger C, Preller KH, Vonmoos M, Jenni D, Bendrick K, Baumgartner MR, Seifritz E, Quednow BB. Altered social and non-social decision-making in recreational and dependent cocaine users. Psychol Med. 2014;44(5):1015-28.

Hulka LM, Vonmoos M, Preller KH, Baumgartner MR, Seifritz E, Gamma A, Quednow BB. Changes in cocaine consumption are associated with fluctuations in self-reported impulsivity and gambling decision-making. Psychol Med. 2015;45(14):3097-110.

Ingram RE, Luxton DD. Vulnerability-Stress Models. In: Hankin BL, Abela JRZ, editors. Development of Psychopathology: A vulnerability stress perspective. Thousand Oaks, CA: Sage Publications Inc.; 2005. p. 32-46.

Karahalil B, Coskun E, Emerce E. ADRA2A polymorphism and smoking in a Turkish population. Toxicol Ind Health. 2008;24(3):171-6.

Kassel JD, Bornovalova M, Mehta N. Generalized expectancies for negative mood regulation predict change in anxiety and depression among college students. Behav Res Ther. 2007;45(5):939-50.

Kaufman J, Charney D. Comorbidity of mood and anxiety disorders. Depress Anxiety. 2000;12 Suppl 1:69-76.

Kelsey RM, Alpert BS, Dahmer MK, Krushkal J, Quasney MW. Alpha-adrenergic receptor gene polymorphisms and cardiovascular reactivity to stress in Black adolescents and young adults. Psychophysiology. 2012;49(3):401-12.

Kendler KS, Karkowski LM, Prescott CA. Causal relationship between stressful life events and the onset of major depression. A J Psychiatry. 1999;156(6):837-41.

Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry. 2005;62(6):593-602.

Keysers C, Kaas JH, Gazzola V. Somatosensation in social perception. Nat Rev Neurosci. 2010;11(6):417-28.

Kim HS, Sherman DK, Mojaverian T, Sasaki JY, Park J, Suh EM, Taylor SE. Gene–culture interaction: Oxytocin receptor polymorphism (OXTR) and emotion regulation. Soc Psychol Personal Sci. 2011;2(6):665-72.

Kim S, Bobeica I, Gamo NJ, Arnsten AF, Lee D. Effects of alpha-2A adrenergic receptor agonist on time and risk preference in primates. Psychopharmacology (Berl). 2012;219(2):363-75.

Kirby KN, Petry NM. Heroin and cocaine abusers have higher discount rates for delayed rewards than alcoholics or non-drug-using controls. Addiction. 2004;99(4):461-71.

Koob GF. Dynamics of neuronal circuits in addiction: reward, antireward, and emotional memory. Pharmacopsychiatry. 2009;42 Suppl 1:S32-41.

Koster EH, Crombez G, Verschuere B, De Houwer J. Selective attention to threat in the dot probe paradigm: differentiating vigilance and difficulty to disengage. Behav Res Ther. 2004;42(10):1183-92.

Kraaij V, Garnefski N, Van Gerwen L. Cognitive coping and anxiety symptoms among people who seek help for fear of flying. Aviat Space Enviro MD. 2003;74(3):273-7.

Krall SC, Rottschy C, Oberwelland E, Bzdok D, Fox PT, Eickhoff SB, Fink GR, Konrad K. The role of the right temporoparietal junction in attention and social interaction as revealed by ALE meta-analysis. Brain Struct Funct. 2015;220(2):587-604.

Kuhn S, Gallinat J. A quantitative meta-analysis on cue-induced male sexual arousal. The journal of sexual medicine. 2011;8(8):2269-75.

Labouebe G, Lomazzi M, Cruz HG, Creton C, Lujan R, Li M, Yanagawa Y, Obata K, Watanabe M, Wickman K, Boyer SB, Slesinger PA, Luscher C. RGS2 modulates coupling between GABAB receptors and GIRK channels in dopamine neurons of the ventral tegmental area. Nat Neurosci. 2007;10(12):1559-68.

Lang PJ. The emotion probe. Studies of motivation and attention. Am Psychol. 1995;50(5):372-85.

Lang PJ, Bradley MM, Cuthbert BN. International affective picture system (IAPS): Affective ratings of pictures and instruction manual. Technical Report A-8. . Gainesville, FL: 2008.

Lazarus RS. From psychological stress to the emotions: a history of changing outlooks. Annu Rev Psychol. 1993;44:1-21.

Lee SJ, Levounis P. Gamma hydroxybutyrate: an ethnographic study of recreational use and abuse. J Psychoactive Drugs. 2008;40(3):245-53.

Lehrl S. Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B) (Multiple Choice Vocabulary Intelligence Test). Göttingen: Hogrefe; 1999.

Levy MN, Blattberg B. The influence of cocaine and desipramine on the cardiac responses to exogenous and endogenous norepinephrine. Eur J Pharmacol. 1978;48(1):37-49.

Li T, Zhu X, Wu X, Li J, Pan L, Li P, Xin Z, Gu HF, Liu Y. Evaluation of the association between the ADRA2A genetic polymorphisms and type 2 diabetes in a Chinese Han population. Genet Test Mol Biomarkers. 2012;16(12):1424-7.

Lynch JC, Mountcastle VB, Talbot WH, Yin TC. Parietal lobe mechanisms for directed visual attention. J Neurophysiol. 1977;40(2):362-89.

MacKillop J. Integrating behavioral economics and behavioral genetics: delayed reward discounting as an endophenotype for addictive disorders. J Exp Anal Behav. 2013;99(1):14-31.

Madea B, Musshoff F. Knock-out drugs: their prevalence, modes of action, and means of detection. Dtsch Arztebl Int. 2009;106(20):341-7.

Maestu J, Villa I, Parik J, Paaver M, Merenakk L, Eensoo D, Harro M, Harro J. Human adrenergic alpha 2A receptor C-1291G polymorphism leads to higher consumption of sweet food products. Mol Psychiatry. 2007;12(6):520-1.

Maier SF, Seligman MEP. Learned helplessness: theory and evidence. J Exp Psychol. 1976;105:3-46.

Mardaga S, Hansenne M. Autonomic aspect of emotional response in depressed patients: relationships with personality. Neurophysiol Clin. 2009;39(4-5):209-16.

Mazur J. An adjusting procedure for delayed reinforcement. New Jersey: Erlbaum; 1987.

McLaughlin KA, Mennin DS, Farach FJ. The contributory role of worry in emotion generation and dysregulation in generalized anxiety disorder. Behav Res Ther. 2007;45(8):1735-52.

McTeague LM, Lang PJ, Laplante MC, Cuthbert BN, Strauss CC, Bradley MM. Fearful imagery in social phobia: generalization, comorbidity, and physiological reactivity. Biol Psychiatry. 2009;65(5):374-82.

Metzger CD, Walter M, Graf H, Abler B. SSRI-related modulation of sexual functioning is predicted by pre-treatment resting state functional connectivity in healthy men. Arch Sex Behav. 2013;42(6):935-47.

Miller SM. Controllability and human stress: method, evidence and theory. Behav Res Ther. 1979;17(4):287-304.

Mineka S, Kihlstrom JF. Unpredictable and uncontrollable events: a new perspective on experimental neurosis. J Abnorm Psychol. 1978;87(2):256-71.

Mischel W, Ayduk O, Berman MG, Casey BJ, Gotlib IH, Jonides J, Kross E, Teslovich T, Wilson NL, Zayas V, Shoda Y. 'Willpower' over the life span: decomposing self-regulation. Soc Cogn Affect Neurosci. 2011;6(2):252-6.

Miu AC, Vulturar R, Chis A, Ungureanu L, Gross JJ. Reappraisal as a mediator in the link between 5-HTTLPR and social anxiety symptoms. Emotion. 2013;13(6):1012-22.

Moffitt TE, Arseneault L, Belsky D, Dickson N, Hancox RJ, Harrington H, Houts R, Poulton R, Roberts BW, Ross S, Sears MR, Thomson WM, Caspi A. A gradient of childhood self-control predicts health, wealth, and public safety. Proc Natl Acad Sci U S A. 2011;108(7):2693-8.

Mogg K, Bradley BP, de Bono J, Painter M. Time course of attentional bias for threat information in non-clinical anxiety. Behav Res Ther. 1997;35(4):297-303.

Mol N, Baas JM, Grillon C, van Ooijen L, Kenemans JL. Startle potentiation in rapidly alternating conditions of high and low predictability of threat. Biol Psychol. 2007;76(1-2):43-51.

Molenberghs P, Cunnington R, Mattingley JB. Brain regions with mirror properties: a meta-analysis of 125 human fMRI studies. Neurosci Biobehav Rev. 2012;36(1):341-9.

Montorsi F, Perani D, Anchisi D, Salonia A, Scifo P, Rigiroli P, Deho F, De Vito ML, Heaton J, Rigatti P, Fazio F. Brain activation patterns during video sexual stimulation following the administration of apomorphine: results of a placebo-controlled study. Eur Urol. 2003;43(4):405-11.

Moser D, Fridriksson J, Bonilha L, Healy EW, Baylis G, Baker JM, Rorden C. Neural recruitment for the production of native and novel speech sounds. Neuroimage. 2009;46(2):549-57.

Mouras H, Stoleru S, Moulier V, Pelegrini-Issac M, Rouxel R, Grandjean B, Glutron D, Bittoun J. Activation of mirror-neuron system by erotic video clips predicts degree of induced erection: an fMRI study. Neuroimage. 2008;42(3):1142-50.

Myrick H, Brady K. Editorial Review: Current Review of the Comorbidity of Affective, Anxiety, and Substance Use Disorders. Curr Opin Psychiatry. 2003;16(3).

Naragon-Gainey K. Meta-analysis of the relations of anxiety sensitivity to the depressive and anxiety disorders. Psychol Bull. 2010;136(1):128-50.

Nemeth Z, Kun B, Demetrovics Z. The involvement of gamma-hydroxybutyrate in reported sexual assaults: a systematic review. J Psychopharmacol. 2010;24(9):1281-7.

Noreika V, Falter CM, Rubia K. Timing deficits in attention-deficit/hyperactivity disorder (ADHD): evidence from neurocognitive and neuroimaging studies. Neuropsychologia. 2013;51(2):235-66.

O'Neill B, Tilley MR, Gu HH. Cocaine produces conditioned place aversion in mice with a cocaine-insensitive dopamine transporter. Genes Brain Behav. 2013;12(1):34-8.

Palamar JJ, Kiang MV, Storholm ED, Halkitis PN. A Qualitative Descriptive Study of Perceived Sexual Effects of Club Drug Use in Gay and Bisexual Men. Psychol Sex. 2014;5(2):143-60.

Park L, Nigg JT, Waldman ID, Nummy KA, Huang-Pollock C, Rappley M, Friderici KH. Association and linkage of alpha-2A adrenergic receptor gene polymorphisms with childhood ADHD. Mol Psychiatry. 2005;10(6):572-80.

Peña-Sarrionandia A, Mikolajczak M, Gross JJ. Integrating emotion regulation and emotional intelligence traditions: a meta-analysis. Front Psychol. 2015;6:160.

Perez de Los Cobos J, Sinol N, Puerta C, Cantillano V, Lopez Zurita C, Trujols J. Features and prevalence of patients with probable adult attention deficit hyperactivity disorder who request treatment for cocaine use disorders. Psychiatry Res. 2011;185(1-2):205-10.

Peters ML, Godaert GL, Ballieux RE, van Vliet M, Willemsen JJ, Sweep FC, Heijnen CJ. Cardiovascular and endocrine responses to experimental stress: effects of mental effort and controllability. Psychoneuroendocrinology. 1998;23(1):1-17.

Peyron C, Petit JM, Rampon C, Jouvet M, Luppi PH. Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods. Neuroscience. 1998;82(2):443-68.

Preller KH, Ingold N, Hulka LM, Vonmoos M, Jenni D, Baumgartner MR, Vollenweider FX, Quednow BB. Increased sensorimotor gating in recreational and dependent cocaine users is modulated by craving and attention-deficit/hyperactivity disorder symptoms. Biol Psychiatry. 2013;73(3):225-34.

Prestes AP, Marques FZ, Hutz MH, Roman T, Bau CH. Tobacco smoking and the ADRA2A C-1291G polymorphism. J Neural Transm. 2007;114(11):1503-6.

Pryce CR, Azzinnari D, Spinelli S, Seifritz E, Tegethoff M, Meinlschmidt G. Helplessness: a systematic translational review of theory and evidence for its relevance to understanding and treating depression. Pharmacol Ther. 2011;132(3):242-67.

Quednow BB, Kuhn KU, Hoenig K, Maier W, Wagner M. Prepulse inhibition and habituation of acoustic startle response in male MDMA ('ecstasy') users, cannabis users, and healthy controls. Neuropsychopharmacology. 2004;29(5):982-90.

Quoidbach J, Berry EV, Hansenne M, Mikolajczak M. Positive emotion regulation and well-being: Comparing the impact of eight savoring and dampening strategies. Pers Indiv Dif. 2010;49(5):368-73.

Robinson ES, Eagle DM, Mar AC, Bari A, Banerjee G, Jiang X, Dalley JW, Robbins TW. Similar effects of the selective noradrenaline reuptake inhibitor atomoxetine on three distinct forms of impulsivity in the rat. Neuropsychopharmacology. 2008;33(5):1028-37.

Roman T, Polanczyk GV, Zeni C, Genro JP, Rohde LA, Hutz MH. Further evidence of the involvement of alpha-2A-adrenergic receptor gene (ADRA2A) in inattentive dimensional scores of attention-deficit/hyperactivity disorder. Mol Psychiatry. 2006;11(1):8-10.

Roman T, Schmitz M, Polanczyk GV, Eizirik M, Rohde LA, Hutz MH. Is the alpha-2A adrenergic receptor gene (ADRA2A) associated with attention-deficit/hyperactivity disorder? Am J Med Genet B Neuropsychiatr Genet. 2003;120B(1):116-20.

Rosler M, Retz W, Retz-Junginger P, Thome J, Supprian T, Nissen T, Stieglitz RD, Blocher D, Hengesch G, Trott GE. [Tools for the diagnosis of attention-deficit/hyperactivity disorder in adults. Self-rating behaviour questionnaire and diagnostic checklist]. Nervenarzt. 2004;75(9):888-95.

Rounsaville BJ. Treatment of cocaine dependence and depression. Biol Psychiatry. 2004;56(10):803-9.

Rude SS, McCarthy CT. Emotional functioning in depressed and depression-vulnerable college students. Cognition and Emotion. 2003;17(5):799-806.

Salomons TV, Johnstone T, Backonja MM, Davidson RJ. Perceived controllability modulates the neural response to pain. J Neurosci. 2004;24(32):7199-203.

Scerbo AS, Freedman LW, Raine A, Dawson ME, Venables PH. A major effect of recording site on measurement of electrodermal activity. Psychophysiology. 1992;29(2):241-6.

Schmid Y, Hysek CM, Preller KH, Bosch OG, Bilderbeck AC, Rogers RD, Quednow BB, Liechti ME. Effects of methylphenidate and MDMA on appraisal of erotic stimuli and intimate relationships. Eur Neuropsychopharmacol. 2015;25(1):17-25.

Schmidt-Daffy M. Modeling automatic threat detection: development of a face-in-the-crowd task. Emotion. 2011;11(1):153-68.

Schmidt CF, Degonda N, Luechinger R, Henke K, Boesiger P. Sensitivity-encoded (SENSE) echo planar fMRI at 3T in the medial temporal lobe. Neuroimage. 2005;25(2):625-41.

Schmitz M, Denardin D, Silva TL, Pianca T, Roman T, Hutz MH, Faraone SV, Rohde LA. Association between alpha-2a-adrenergic receptor gene and ADHD inattentive type. Biol Psychiatry. 2006;60(10):1028-33.

Sehlmeyer C, Dannlowski U, Schoning S, Kugel H, Pyka M, Pfleiderer B, Zwitserlood P, Schiffbauer H, Heindel W, Arolt V, Konrad C. Neural correlates of trait anxiety in fear extinction. Psychol Med. 2011;41(4):789-98.

Seligman MEP. Helplessness. San Francisco: Freeman; 1975.

Sewards TV, Sewards MA. Representations of motivational drives in mesial cortex, medial thalamus, hypothalamus and midbrain. Brain Res Bull. 2003;61(1):25-49.

Snead OC, 3rd, Gibson KM. Gamma-hydroxybutyric acid. N Engl J Med. 2005;352(26):2721-32.

Song AW, Huettel SA, McCarthy G. Functional neuroimaging: Basic principles of functional MRI. In: Cabeza R, Kingstone A, Cabeza R, Kingstone A, editors. Handbook of functional neuroimaging of cognition (2nd ed). Cognitive neuroscience. Cambridge, MA, US: MIT Press; 2006. p. 21-52.

Stevenson J, Langley K, Pay H, Payton A, Worthington J, Ollier W, Thapar A. Attention deficit hyperactivity disorder with reading disabilities: preliminary genetic findings on the involvement of the ADRA2A gene. J Child Psychol Psychiatry. 2005;46(10):1081-8.

Stoleru S, Fonteille V, Cornelis C, Joyal C, Moulier V. Functional neuroimaging studies of sexual arousal and orgasm in healthy men and women: a review and meta-analysis. Neurosci Biobehav Rev. 2012;36(6):1481-509.

Thomas E, Dewald L. Experimental neurosis. In: Seligman MEP, editor. Psychopathology:

Experimental models. San Francisco: Freeman; 1977.

Thompson RA. Emotion regulation: a theme in search of definition. Monogr Soc Res Child Dev. 1994;59(2-3):25-52.

Thorell LH. Valid electrodermal hyporeactivity for depressive suicidal propensity offers links to cognitive theory. Acta Psychiatr Scand. 2009;119(5):338-49.

Toledano R, Pfaus J. The Sexual Arousal and Desire Inventory (SADI): a multidimensional scale to assess subjective sexual arousal and desire. J Sex Med. 2006;3(5):853-77.

Tull MT, Roemer L. Emotion regulation difficulties associated with the experience of uncued panic attacks: Evidence of experiential avoidance, emotional nonacceptance, and decreased emotional clarity. Behavior Therapy. 2007;38(4):378-91.

Urcelay GP, Dalley JW. Linking ADHD, impulsivity, and drug abuse: a neuropsychological perspective. Curr Top Behav Neurosci. 2012;9:173-97.

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002;3(7):RESEARCH0034.

Volkow ND, Wang GJ, Fowler JS, Telang F, Jayne M, Wong C. Stimulant-induced enhanced sexual desire as a potential contributing factor in HIV transmission. A J Psychiatry. 2007;164(1):157-60.

Vonmoos M, Hulka LM, Preller KH, Jenni D, Baumgartner MR, Stohler R, Bolla KI, Quednow BB. Cognitive dysfunctions in recreational and dependent cocaine users: role of attention-deficit hyperactivity disorder, craving and early age at onset. Br J Psychiatry. 2013;203(1):35-43.

Vonmoos M, Hulka LM, Preller KH, Minder F, Baumgartner MR, Quednow BB. Cognitive impairment in cocaine users is drug-induced but partially reversible: evidence from a longitudinal study. Neuropsychopharmacology. 2014;39(9):2200-10.

Wagner R. Tristan und Isolde Leipzig: Breitkopf & Härtel; 1860.

Walter M, Bermpohl F, Mouras H, Schiltz K, Tempelmann C, Rotte M, Heinze HJ, Bogerts B, Northoff G. Distinguishing specific sexual and general emotional effects in fMRI-subcortical and cortical arousal during erotic picture viewing. NeuroImage. 2008;40(4):1482-94.

Walter M, Stadler J, Tempelmann C, Speck O, Northoff G. High resolution fMRI of subcortical regions during visual erotic stimulation at 7 T. MAGMA. 2008;21(1-2):103-11.

Washio Y, Higgins ST, Heil SH, McKerchar TL, Badger GJ, Skelly JM, Dantona RL. Delay discounting is associated with treatment response among cocaine-dependent outpatients. Exp Clin Psychopharmacol. 2011;19(3):243-8.

Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. J Pers Soc Psychol. 1988;54(6):1063-70.

Webb TL, Miles E, Sheeran P. Dealing with feeling: A meta-analysis of the effectiveness of strategies derived from the process model of emotion regulation. Psychol Bull. 2012;138(4):775-808.

Weiss NH, Tull MT, Davis LT, Dehon EE, Fulton JJ, Gratz KJ. Examining the association between emotion regulation difficulties and probable posttraumatic stress disorder within a sample of African Americans. Cog Behav Ther. 2012;41(1):5-14.

Wiech K, Kalisch R, Weiskopf N, Pleger B, Stephan KE, Dolan RJ. Anterolateral prefrontal cortex mediates the analgesic effect of expected and perceived control over pain. J Neurosci. 2006;26(44):11501-9.

Wirtz CM, Hofmann SG, Riper H, Berking M. Emotion regulation predicts anxiety over a five-year interval: A cross-lagged panel analysis. Depress Anxiety. 2014;31(1):87-95.

Wittchen HU, Wunderlich U, Gruschwitz S, Zaudig M. SKID-I. Strukturiertes Klinisches Interview für DSM-IV Achse I: Psychische Störungen. [SCID-I. Structured Clinical Interview for DSM-IV Axis I Disorders]: Hogrefe; 1997.

Wolpe JM. Psychotherapy by reciprocal inhibition. Stanford: Stanford University Press; 1958.

Zvolensky MJ, Lejuez CW, Eifert GH. Prediction and control: operational definitions for the experimental analysis of anxiety. Behav Res Ther. 2000;38(7):653-63.

8 Acknowledgments

I would like to express my gratitude to those who have contributed to this thesis with their guidance and support.

First, I would like to thank my supervisor and mentor, Prof. Dr. med. Erich Seifritz, for his consistent support and guidance. He bestowed upon me the opportunity to work in an excellent scientific environment, and provided me with the right amount and balance of freedom and stability to flourish.

Secondly, I would like to specially thank my supervisor and mentor, Prof. Dr. Boris Quednow, for teaching me how to accurately and efficiently conduct research. He was not involved in this PhD project from the beginning, but he nevertheless generously supported me with his experience and guidance, and we finally published four fascinating papers together.

Many words of appreciation are owed to PD Dr. Chris Pryce for his advice and support in organizing my PhD and for his important contribution to study A, Dr. med. Milan Scheidegger for his valuable advice during my progress and for teaching me the fMRI analysis for study B, Prof. Dr. Willibald Ruch for his support and assistance in my gelotophobia study, and of course Prof. Dr. Klaas Prüssmann for supervising and overseeing my PhD.

I would also like to extend thanks to Sara Romer for her invaluable assistance in the practical execution of all of my studies, and for helping me tremendously in recruiting participants and gathering data.

I would like to thank all my appreciated collaborators, such as Dr. med. Oliver Bosch, Dr. Matthias VonMoos, Dr. Philipp Stämpfli, PD Dr. Edna Grünblatt, Dr. med. Josef Hättenschwiler, Dr. med. Maximilian Buschner, Dr. med. Ramin Mansour, Dr. med. Thomas Chmielewski, and Dr. Katharina Gaudlitz.

Last, but not least, I would like to particularly thank my parents, my brother, and my girlfriend for supporting and inspiring me along the way.

9 Curriculum Vitae

Michael Marco HAVRANEK, MD

Contact and Personal Information

Address	Seestrasse 58
	CH - 6052 Hergiswil
Telephone	+41/ 41 632 52 58
Email	m.havranek@bli.uzh.ch
Date of birth	21.12.1985
Place of birth	Lucerne, LU
Nationality	CH

Education and Training

2010 - 2013	Bachelor in Psychology, University of Zurich
2006 - 2010	Master in Biology (MD/PhD Program), University of Zurich
2004 - 2010	Bachelor and Master in Medicine, University of Zurich and Ludwig-Maximilians University of Munich, Germany (Graduation in 2010)
1998 - 2004	Matura in Modern Languages, Kollegium St. Fidelis, Stans (Graduation in 2004)
1992 - 1998	Primary School, Hergiswil

Research and Clinical Experience

2011 - 2015	PhD Thesis at the Psychiatric University Hospital Zurich
2009	Research Internship at the Laboratory for Neurology and Imaging of Cognition, University of Geneva
2008 - 2009	Clinical Internships in Internal Medicine (Kantonsspital Heiden), Neurology (Kantonsspital Aarau), Neurosurgery (Kantonsspital Aarau), Neuropediatrics (University Hospital Zurich) and Neuroradiology (University Hospital Zurich)
2007 - 2013	MD Thesis at the Neuropsychological institute, University of Zurich
2006	Research Internship at the Institute of Neuropathology, University Hospital Zurich
2005 - 2006	Research Internship at the Institute of Molecular Cancer Research, University of Zurich

Publications

Havranek M, Langer N, Cheetham M, Jäncke L. Perspective and agency during video gaming influences spatial presence experience and brain activation patterns. Behav Brain Funct 2012; 8:34.

Easton AC, Lourdusamy A, **Havranek M,** Mizuno K, Müller CP. αCaMKII controls the establishment of cocaine's reinforcing effects in mice and humans. Transl Psychiatry 2014;4:e457.

Havranek MM, Bolliger B, Roos S, Pryce CR, Quednow BB, Seifritz E. Uncontrollable and unpredictable stress interacts with subclinical depression and anxiety scores in determining anxiety response. Stress. In press.

Bosch OG, **Havranek MM,** Baumberger A, Preller KH, VonRotz R, Herdener M, Kraehenmann R, Stämpfli P, Scheidegger M, Seifritz E, Quednow BB. Neural underpinnings of prosexual effects induced by gamma-hydroxybutyrate in healthy humans. In submission.

Havranek MM, Hulka LM, Tasiudi E, Eisenegger C, Vonmoos M, Preller KH, Mössner R, Baumgartner MR, Seifritz E, Grünblatt E, Quednow BB. α2A-adrenergic receptor polymorphisms and mRNA expression levels are associated with delay discounting in cocaine users. Addict Biol. In press.

Havranek MM, Volkart F, Bolliger B, Roos S, Buschner M, Mansour R, Chmielewski T, Gaudlitz K, Hättenschwiler J, Seifritz E, Ruch W. The fear of being laughed at as a diagnostic criterion in social anxiety disorder and avoidant personality disorder. In submission.

Havranek MM, Vonmoos M, Müller CP, Büetiger JR, Tasiudi E, Hulka LM, Preller KH, Mössner R, Grünblatt E, Seifritz E, Quednow BB. Serotonin transporter and tryptophan hydroxylase gene variations mediate working memory deficits of cocaine users. Neuropsychopharmacology. 2015. doi: 10.1038/npp.2015.146.

Declaration of Originality

I hereby declare that this thesis represents my original work and that I have used no other sources except as noted by citations. All data, tables, figures and text citations, which have been reproduced from any other source, including the internet, have been explicitly acknowledged as such.

Michael M. Havranek, matriculation number: 04-717-450

Place, date Signature

Zürich, 14.12.2015